2017

Characterization of Pyrenophora Tritici-Repentis in Wheat and Rye to Study Tan Spot Susceptibility and Insights Into Its Relationship with Stem Rust Resistance

Sidrat Abdullah
South Dakota State University

CHARACTERIZATION OF *PYRENOPHORA TRITICI-REPENTIS* IN WHEAT AND RYE TO STUDY TAN SPOT SUSCEPTIBILITY AND INSIGHTS INTO ITS RELATIONSHIP WITH STEM RUST RESISTANCE

BY

SIDRAT ABDULLAH

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Plant Science

South Dakota State University

2017

CHARACTERIZATION OF PYRENOPHORA TRITICI-REPENTIS IN WHEAT AND RYE TO STUDY TAN SPOT SUSCEPTIBILITY AND INSIGHTS INTO ITS RELATIONSHIP WITH STEM RUST RESISTANCE

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Plant Science degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidates are necessarily the conclusions of major department.

Shaukat Ali, Ph.D. Date
Thesis Advisor

David Wright, Ph.D. Date
Head, Department of Agronomy,
Horticulture, and Piant Science (AHPS)

Dean, Graduate School Date

ACKNOWLEDGEMENTS

First of all, I would like to give heartfelt thanks to Almighty by the grace of whom I got an opportunity to start my Ph.D. study and make it happens successfully. I would like to give deepest gratitude to my advisor Dr. Shaukat Ali for providing me an opportunity to work in this project as a Ph.D. student. I appreciate him for assisting in design, supervision during conducting experiments, as well as valuable inputs and suggestions during the preparation of this dissertation.

I would like to express my deep gratidue to Dr. Sunish Kumar Sehgal one of my committee members for his immense support throughout my laboratory work. His valuable comments and guidance helped me a lot to sum up the dissertation in time.

I would also like to thank the members of my graduate committee: Dr. Marie Langham, Dr. Byamukama Emmanuel for their valuable comments, suggestions and corrections during my research as well as in this dissertation.

I would like to give special thanks to Dr. Rocky Dailey Graduate Faculty Representative, for his kind consent to be a part of my committee and his regular attendance in each meeting during the different stages of my research and delivering his comments.

I acknowledge Dr. Jai Rohila, a former member of my committee, and Dr. Liu Zhaohui at North Dakota State University for guiding me in the molecular part of this study as well as allowing me to work in their laboratorties. I would also like to thank Dr. Steve Meinhart for his assistance with the protein purification procedure in his laboratory at North Dakota State University.

I do appreciate and am grateful to Mr. Richard Geppert for his assistance in the field during leaf samples collection from different counties of South Dakota and cooperation during laboratory and greenhouse work especially for multiplication of *Pyrenophora tritici-repentis* isolates and growing cultures. I am also very thankful to Dr. Moustafa Eldakak for training and guiding me in molecular work at the beginning of my study, and I'm also grateful to my lab mate, Navjot Kaur, for her continuous assistance in the lab work and data recording thoughout my research. I am also very much thankful to Sidhu Jagdeep Singh for helping me out making a map of geographical locations. My appreciation also goes to Girma Tadesse Ayana for providing assistance when running the statistical software.

My humble thanks and gratitude are due to whole Plant Science Department staff, friends, and for those who contributed to make this dissertation possible.

It is an honour for me to acknowledge 'Department of Agronomy, Horticulture, Plant Science' for financial assistance to pursue a Ph.D. at South Dakota State University (SDSU), Brookings, SD. It is my pleasure to extend gratitude to my organization, Bangladesh Agricultural Research Institute (BARI) for granting me deputation for higher studies abroad.

Last but not least, I always remember my beloved late father, and I'm grateful to my mother and sister for their patience and encouragement during this study. My sincere thanks also go to my wife for her inspiration, patience and maintaining the home throughout the entire study. Finally, thanks are given to my cute daughter, Sarrinah Abdullah, for adding happy moments in my life besides this study.

CONTENTS

LIST OF FIGURES

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES

ABSTRACT

CHARACTERIZATION OF *PYRENOPHORA TRITICI-REPENTIS* IN WHEAT AND RYE FOR STUDY OF TAN SPOT SUSCEPTIBILITY AND INSIGHTS INTO ITS RELATIONSHIP WITH STEM RUST RESISTANCE

SIDRAT ABDULLAH

2017

Tan spot, caused by the ascomycete fungus, *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar fungal disease of wheat worldwide. To date, *Ptr* isolates have been grouped into eight races based on the production of three host selective effectors (toxins), Ptr ToxA, ToxB, and ToxC, which are associated with necrosis and chlorosis symptoms on wheat differential lines. In order to see the correlation between stem rust resistance and tan spot susceptibility in 1970's, to know the pathogen virulence profile, and explore the sources of tan spot resistance, we recovered isolates from wheat and rye and evaluated wheat and rye lines with different *Ptr* isolates. To determine the reaction of 247 pre-stem rust-epidemic wheat genotypes (n=156 bread and n=91 durum) against stem rust (TTKSK) and tan spot *Ptr* race1 (SD-13-101) and host-specific toxin Ptr ToxA, we observed 69% of the both bread and durum wheat cultivars were susceptible to both tan spot and stem rust. Thirty-nine percent of both bread and durum wheat genotypes gave differential responses (Susceptible/insensitive and Resistance/Sensitive) to race1 and Ptr ToxA respectively. Through genotyping 903 *Ptr* isolates we found majority of the islates with *ToxA* gene. A few isolates had the *ToxB* gene. Of the 903 isolates recovered, 273 were phenotyped for their race characterization using the wheat differential set.

About 77 % of the isolates were designated as race 1, whereas 41% were identified as race 4. Most of the race 4 isolates were recovered from rye (77%). Only 4.3% isolates (one from SD and eleven from Baltic region) were grouped as race 2 and two isolates were classified as race 5 from SD. Twenty-four isolates identified which induced necrosis and chlorosis in Glenlea (ToxA sensitive) and 6B365 respectively or necrosis only in Glenlea but did not harbor the *ToxA* gene. Rye lines (39%) showed more susceptibility to race 5 when compared to race 1(21%) and were insensitive to both Ptr ToxA and Ptr ToxB. Based on our study, no correlation of stem rust resistance and increase in tan spot occurrence was observed. Similarly, Ptr ToxA is not the only factor responsible for the increase of tan spot disease in 1970's and beyond. Our results show races 1 and 4 are largely prevalent on rye in the region with high frequency of race 4 as those isolates are avrulent on wheat suggesting its minimal role in the disease epidemiology. Rye could serve as a good source of resistance if needed. In terms of diversity of *Ptr* isolates, results of our study indicate that a wide range of virulence variation profile present in wheat in South Dakota, Baltic region and Romania, and some of the *Ptr* isolates from SD, Lithuania and Romania which did not harbor *ToxA* and did not fit into the currently prevalent 8 races. *Ptr* race 5, observed for the first time from SD.

CHAPTER 1

GENERAL INTRODUCTION

Tan spot of wheat and economic importance

Tan spot of wheat, is one of the most important foliar fungal diseases in wheat growing regions worldwide (Hosford, 1982). It is caused by the fungus, *Pyrenophora tritici-repentis* (Died.) Drechs (anamorph: *Drechslera tritici-repentis* (Died.) Shoem., and produces lens shaped necrotic spots delimited by chlorotic halo symptoms in susceptible cultivars. The pathogen can attack durum (*Triticum turgidum* L. var durum) and bread wheat (*Triticum aestivum* L.), as well as many other cereals like barley, oats, rye and various non-cereal grass species (Ali and Francl, 2003; Hosford, 1971b; Krupinsky, 1982; 1992a). In USA, tan spot was first observed on wheat in New York, in 1940 and later in other states but it was considered a minor disease (DeWolf et al., 1998). The disease became economically important for wheat production starting from mid-1970's in major wheat growing countries worldwide (Hosford, 1974, 1982; Kohli et al., 1992; Rees and Platz, 1979). The first serious tan spot outbreak was observed in Canada in 1974 (Tekauz, 1976). The disease was also observed with high incidence and severity throughout the southern region of South America: Argentina, Brazil, Chile, Paraguay and Uruguay (Kohli et al., 1992). Yield losses due to tan spot have been reported up to 50% depending on the cultivar susceptibility level and disease severity in USA (Shabeer and Bockus, 1988). In Germany, drop in grain yield owing to tan spot ranged from 10-36% (Wolf and Hoffmann, 1993). Further, yield losses due to the disease may vary depending upon the crop growth stage at the time of infection: 13 % yield

reduction occurs during the early growth stage (seedling) disease infection, 35% during late growth stage (milk stage) stage and 48% from presence of disease throughout the season (Reez and Platz, 1983). The losses are not only due to yield reduction but the fungus also impacts the seed quality by causing red and dark smudge (Fernandez et al., 2001). Tan spot can be managed by using fungicides, crop rotation with non-cereals, and resistant cultivars (De Wolf et al., 1998; Singh et al., 2010). The use of resistant cultivars is environment friendly and generally an economically viable means for tan spot management.

Disease origin and distribution

Tan spot caused by *P. tritici-repentis* was first reported in wheat from Japan in 1928 (Nisikado, 1928). Later, the disease was reported from India in 1934 (Mitra, 1934), Canada in 1937 (Conners, 1937), the USA in 1940 (Barrus and Johnson, 1942), Australia in 1953 (Shaw and Valder, 1953) and Kenya in 1954 (Duff, 1954). The following synonyms: yellow leaf blotch, yellow leaf spot, eye spot and leaf blight of tan spot have been used since the disease was reported on wheat (Hosford, 1982). Though the disease was observed during the 1930's in North America, but it was not considered as a serious threat to wheat production until the early 1970's when it became widespread in the Australia, Canada, and USA (Hosford, 1982). Rapid spread of tan spot in the wheat growing areas of North America and elsewhere is thought to be due to : 1) practicing zero or minimal tillage at the farm level which helps the fungus overwinter from one season to the next growing season; 2) unintentionally increasing tan spot susceptibility in wheat cultivars while deploying stem rust resistance genes for combating stem rust

epidemics which occurred in the 1950's (Hosford, 1982; Lamari et al., 2005b; Strelkov and Lamari, 2003); and 3) change in the pathogen virulence, such as gaining *Ptr ToxA* gene, from *Stagonospora nodorum* in 1940's through horizontal gene transfer. The *PtrToxA* gene produces a host-selective toxin "Ptr ToxA" responsible for tan spot symptom in the toxin sensitive wheat genotypes (Friesen et al., 2006). Further, long distance dispersal of diseased seed during the germplasm exchange among researchers for various purposes could be liable for wide spread occurrence of tan spot worldwide. However, it is still not clear how tan spot became so widespread and damaging in other wheat growing regions in 1970's and onwards.

History of the pathogen and nomenclature

The fungus was recovered for the first time from intermediate grass (*Agropyron repens*) in Germany (Diedicke, 1902). Diedicke (1902) described the fungus and initially named as *Pleospora trichostroma* and later on he called it *Pleospora tritici-repentis* (Diedecke, 1904). After the fungal description given by Diedicke, the fungus has been known by different sexual and asexual names and that include *Pleospora tritici-repentis* Died*, Pleospora trichostroma f. species tritici-repentis* (Died.) Noack, *Pyrenophora tritici-repntis* (Died.) Drechs. and *Pyrenophora tritici-vulgaris* Dickson for the fungal sexual state; whereas, *Helminthosporium gramineum* Rab. Ex Schlecht f. sp. *tritici-repentis* Died, *H. tritici-repentis* (Died.) Died, *Helminthosporium tritici-vulgaris* Nisikado Ito, *Drechslera tritici-repentis* (Died.) Shoem. are names used for the fungal asexual state. Presently, *Pyrenophora tritici-repentis* (Died.) Drechs. and *Drechslera tritici-repentis*

(Died.) Drechs. names are globally used and recognized for the fungal sexual and asexual state, respectively (DeWolf et al., 1998).

Pathogen biology and life cycle

Pyrenophora tritici-repentis (Died) Drechs. is a homothallic fungus (Hosford, 1971a; Shoemaker, 1962). The fungus undergoes both sexual and asexual reproduction by producing ascospores and conidiospores, respectively (Shoemaker, 1962). For overwintering in the Northern Great Plains, the fungus survives on infected wheat debris lying on the soil surface and produce one-loculed black raised fruiting bodies (pseudothecia) approximately 0.2-0.35 mm in diameter (Hosford, 1972) (Figure 1.1). The pseudothecia contain asci where each ascus produces 8 ascospores (Hosford, 1971a; Shoemaker, 1962). The asci are paired walls with pseudoparaphysis between the asci. The center cell of an ascospore has a longitudinal septation with 4-5 cells (Ellis and Waller, 1976). The fungal conidiophores are olive-black with an inflated base, and the conidia are hyaline and cylindrical shaped with 4-6 septa. The conidia have conically-tapered basal cells (snake head) (Shoemaker, 1962).

The fungus overwinters on crop residue in the form of sexual fruiting body, pseudothecia, that produce ascospores. The ascospores serve as the primary source of inoculum for the next cropping season. Additional sources of primary inoculum include infected seed; conidia formed on infested crop residues; alternative grass hosts; and volunteer wheat plants (Schilder and Bergstrom, 1993). Very rarely conidia develop on pseudothecia also have been observed (Shoemaker, 1962). Tan spot lesions developed on seedlings can produce conidia in multiple cycles under suitable weather conditions,

serving as secondary inoculum sources. The conidia are spread via wind within the field and can travel long distance as far as 100 km (Francl, 1997).

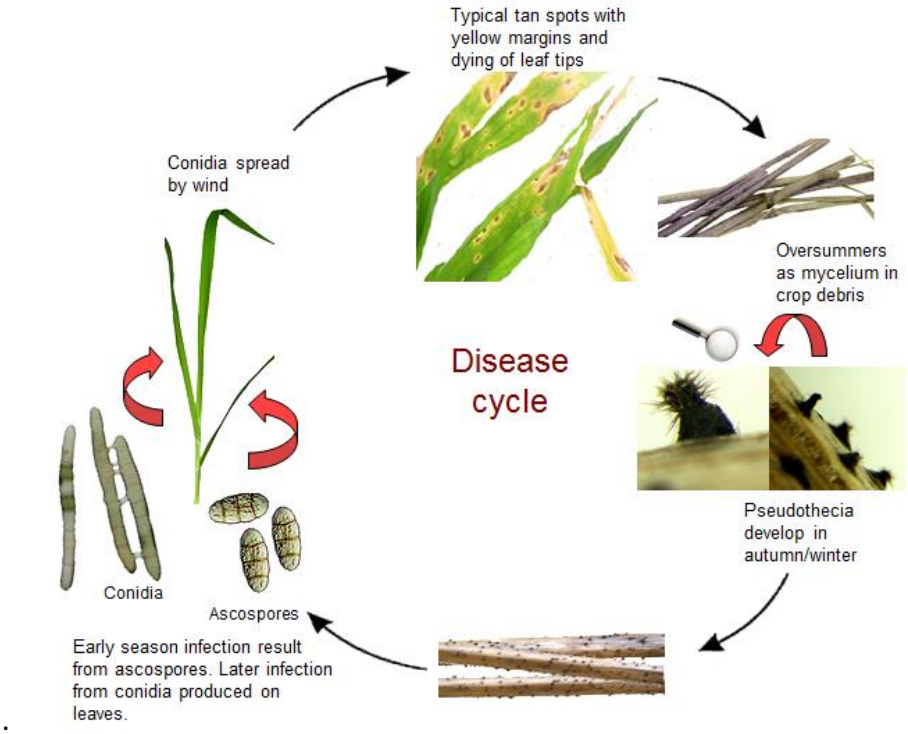

Figure 1.1. Tan spot of wheat disease cycle (Manisha, 2016)

The infection process

During the spring season the ascospore or conidium of *P. tritici-repentis* lands on the wheat leaf, germinates, produces germ tube, and forms an appressorium and penetration peg that help the fungus in direct penetration into epidermal cells (Larez et al., 1986). The conidial germination rate and appressorium formation depends on the environmental conditions (Amaike et al., 2008; Larez et al., 1986). Cool weather temperatures above 10°C (50°F) and frequent spring rains with high relative humidity influence/favor the rate of germ tube multiplication from conidia and ascospores

(Hosford et al., 1987). During the fungal penetration process, both mechanical pressure and enzymatic reactions are involved (Dushnicky et al., 1996). When the germ tube forms the appressorium, just beneath a penetration peg is formed which allows the fungus to enter directly or through stomata forming a vesicle (Dushnicky et al., 1998a). The whole penetration process is normally completed within 3 hours after landing the spore on the leaf surface (Dushnicky et al., 1996). The fungus often develops an intracellular vesicle after penetration from the penetration peg (Dushnicky et al., 1996; Dushnicky et al., 1998a; Larez et al., 1986). In some cases, no vesicle is formed but hyphae can still go through the epidermal surface (Larez et al., 1986). In the early phases of infection, *P. tritici-repentis* assaults both susceptible and resistant hosts and causes intercellular injury (Larez et al., 1986). Conversely, the papillae number formed in resistant cultivars were higher than in susceptible ones (Dushnicky et al., 1996; Dushnicky et al., 1998a). In resistant genotypes, fungal invasion of the mesophyll is clogged, causing the growth of a few small spots or lesions on the leaves (Dushnicky et al., 1996; Dushnicky et al., 1998b). On the other hand, in susceptible cultivars the hyphae continuously grow through the mesophyll cells and finally develop into a large brown lens-shaped necrotic lesions bordered by a chlorotic halo with a small black point in the center (Figure 1.2) (De Wolfe et al., 1998; Hosford, 1982).

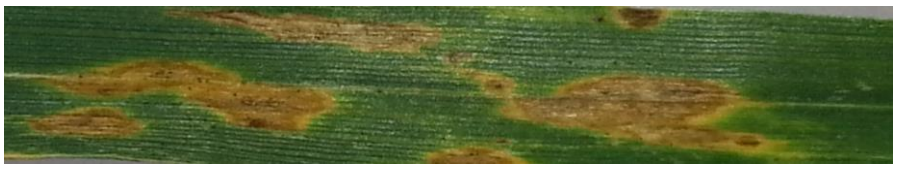

Figure 1.2. Tan spot symptoms, tan necrotic lesion with a chlorotic halo, on spring wheat

Tan spot disease symptoms and *Pyrenophora tritici-repentis* race classification system

The disease symptoms on susceptible wheat plants are large oval or diamond-shaped necrotic and/or chlorotic spots with a pin head size point in the center (Figure 1.2). The lesion may coalesce and cover most of the leaf area under severe infection. The fungus causes a small black spot with no or little brown lesion on the leaf surface of resistant wheat genotypes (Hosford, 1982). Lamari and Bernier (1989b) identified two qualitative types of symptoms, tan necrosis and chlorosis produced by the fungus on susceptible differential wheat genotypes (Figure 1.3). Lamari and Bernier (1989b) classified *P. tritici-repentis* isolates into four pathotypes based on their ability to produce necrosis and/or chlorosis on four differential wheat genotypes Glenlea, 6B662,6B365, and Salamouni.

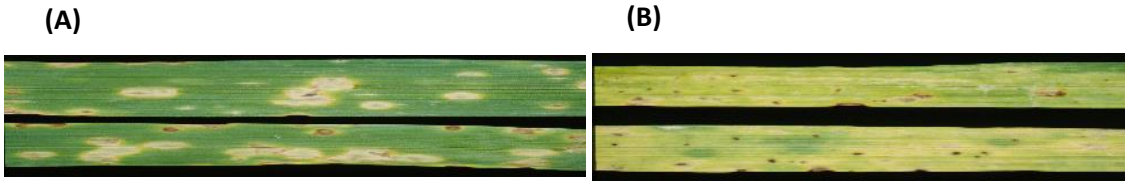

Figure 1.3. Two distinct symptoms produced by *P. tritici-repentis* on wheat cultivar Glenlea inoculated with race 1(A) and chlorosis on wheat line 6B365 inoculated with race 1 (B)

The isolates that produce necrosis on Glenlea and chlorosis on 6B365 were classified as pathotype 1, necrosis only on Glenlea, pathotype 2, chlorosis only on 6B365, pathotype 3, and neither necrosis nor chlorosis on all four differential genotypes, pathotype 4. The differential genotype Salamouni was resistant to all four pathotypes.

Later on, Lamari et al. (1995) collected some *P. tritici-repentis* isolates from Algeria that were comparable to pathotype 3, but they produced chlorosis on 6B662 instead on 6B365 genotype. It was not possible to group these new isolates under pathotype 3. In order to accommodate these new isolates, a race-based classification system was suggested by Lamari et al. (1995); where pathotype 1-4 was designated as races 1- 4, and the isolates with new virulence were designated as race 5 (Table 1.1). At present, eight races have been identified in the fungal population (Lamari et al., 2003). Race 1 (followed by race 2) is the most commonly prevalent race on wheat worldwide (Ali and Francl, 2003; Gamba et al., 2012; Lamari and Strelkov, 2010). Races 3 and 4 are also found infrequently in the USA and Canada (Ali and Francl, 2003; Engle et al., 2006; Lamari and Bernier, 1989a). Races 5 and 6 are observed in Africa (Strelkov et al., 2002); race 5 has also been observed in the USA and Canada (Abdullah et al., 2016; Ali and Francl, 2003; Strelkov et al., 2002) and in Azerbaijan (Lamari et al., 2003). The races 7 and 8 were found to be common in the Caucasus and Fertile Crescent regions. Which signify the center of wheat diversity and may also be a diversity center for *P. tritici-repentis* (Lamari and Strelkov, 2010). Subsequently a long-term host-pathogen coevolution, of almost all the races (1- 8) were observed in these regions (Lamari et al., 2003, 2005b; Strelkov et al., 2002). Three host-selective toxins Ptr ToxA, Ptr ToxB, and Ptr ToxC have been recovered from the fungal populations, and they are associated with necrosis and chlorosis symptoms in toxin sensitive wheat genotypes (Lamari et al., 2003). For designating a new *P. tritici-repentis* race the new race phenotypic reaction should be associated with a new toxin (Cieffuti et al., 1998). At present, researchers

involved in studying tan spot-wheat host-pathogen system globally follow race classification system (Table 1.1). Therefore, some isolates have been identified with different virulence based on their phenotypic reactions, but the toxins associated with this new virulence were not found. Thus, these isolates still await race designation (Ali et al., 2010; Andrie et al., 2005; Lepoint et al., 2010).

Table 1.1. Races of *Pyrenophora tritici-repentis* (Lamari et. al., 2003).

		Wheat differential lines			
Races	**Ptr Toxin Genes**	**Glanlea (Sensitive to ToxA)**	**6B365 (Sensitive to ToxC)**	**6B662 (Sensitive to ToxB)**	**Salamouni (Insensitive to Toxin A, B and C**
Symptom →		**Necrosis** 	**Chlorosis** 	**Chlorosis** 	**Avirulent** 
1	*ToxA* and *ToxC*	Necrosis	Chlorosis	Avirulent	Avirulent
2	*ToxA*	Necrosis	Avirulent	Avirulent	Avirulent
3	*ToxC*	Avirulent	Chlorosis	Avirulent	Avirulent
4	Nil	Avirulent	Avirulent	Avirulent	Avirulent
5	*ToxB*	Avirulent	Avirulent	Chlorosis	Avirulent
6	*ToxB* and *ToxC*	Avirulent	Chlorosis	Chlorosis	Avirulent
7	*ToxA* and *ToxB*	Necrosis	Avirulent	Chlorosis	Avirulent
8	*ToxA, ToxB* and *ToxC*	Necrosis	Chlorosis	Chlorosis	Avirulent

Alternative hosts of *Pyrenophora tritici-repentis*

Any host plant species, other than the principle or economical host plant species, which serves as a host for pathogen survival is known as an alternative host. Alternative hosts could play an important role in fungal reproduction, contribute additional inoculum for disease development, serve as a pathogen reservoir between cropping seasons, and are a source of genetic variability (DeWolf et al., 1998). However, alternative hosts can be good resistance sources (Saulescu et al., 2011; Rabinovich, 1998). The fungus, *P. tritici-repentis,* has been reported to infect many grass species in addition to its economic host, wheat. These alternative hosts include *Hordeum vulgare*, *Avena sativa*, *Secale cereale*, *Agropyron* sp., *Alopecurus arundinaceus*, *Andropogon gerardi*, *Avena fatua*, *A. sativa*, *Bromus inermis*, *Dactilys glomerata*, *Echinochloa* sp., *Elymus innovatus*, *Hordium vulgare*, *Lolium perenne*, *Phalaris arundinaceae*, and *Secale cereale* (Ali & Francl, 2002; Andersen, 1955; Conners, 1939; Dennis and Wakefield, 1946; Dickson, 1956; Diedicke, 1902; Drechsler, 1923; Farr et al., 1989; Hosford, 1971b; Howard & Morral, 1975; Krupinsky, 1992a 1992b; Shoemaker, 1962; Sprague, 1950). Krupinsky (1987) studied twenty-seven *P. tritici-repentis* isolates recovered from smooth bromegrass and tested them on both wheat and smooth bromegrass. He reported that all the isolates were virulent on wheat as well as on smooth bromegrass but the isolates interaction on wheat was different in virulence level. Similar results were observed when Krupinsky (1992a) recovered 62 *P. tritici-repentis* isolates from 25 grass species and found them pathogenic on wheat. Some of the isolates that were recovered from Altai wild rye, Basin wild rye, and Russian wild rye showed variation in aggressiveness.

The isolates evaluated on tan spot-susceptible wheat genotypes in these studies varied significantly in producing tan spot symptoms based on percent necrotic area and lesion length as compared to the isolates recovered from wheat. Krupnisky (1992b) recovered 87 single-spore isolates of *P. tritici-repentis* from various grass species and barley. When these were evaluated for their pathogenicity on wheat, variation in their aggressiveness indicated alternative hosts could be a potential source of genetic variation in the pathogen population. Among the alternative hosts, barley and oats were found less susceptible to tan spot as compared to rye (Hosford, 1971b). In another study by Ali and Francl (2001), ten *P. tritici-repentis* isolates were recovered from barley, and all of them were characterized as race 1. Further, they tested several two and four-row barley cultivars against *P. tritici-repentis* races 1, 2, 3, and 5 and two host-selective Ptr ToxA and Ptr ToxB toxins. All the evaluated cultivars were resistant to the fungus and insensitive to the toxins. They concluded that barley may not have any significant role in tan spot epidemiology. In contrast, 74 barley genotypes were evaluated against races 5, 6, 7, and 8. Two barley genotypes, ('Rivers' and 'Newal') out of the 74 were found susceptible to race 5 and sensitive to Ptr ToxB (Aboukhaddour and Strelkov, 2016). Ali and Francl (2003) evaluated 92 isolates recovered from several non-cereal grasses including smooth bromegrass, intermediate wheatgrass, and crested wheatgrass leaf samples for their race structure. They found only two isolates recovered from smooth bromegrass were race 1, and the rest of them were race 4. They concluded from their results that non-cereal grasses including smooth bromegrass might possess a different *P. tritici-repentis* population pool. Similar results were reported in another independent

study where the fungal isolates recovered from non-cereal grasses were also identified as race 4 (Sarova et al., 2005).

Host Selective Toxins (Effectors) from *P. tritici-repentis* and their role in tan spot development

Several plant pathogens secrete toxic molecules (toxins) that increase the pathogens virulence generally by rapidly killing the host cells. Generally, these toxins are produced by bacterial and fungal necrotrophic pathogens (Alfano and Collmer, 1996; Walton, 1996). The toxins can be distinct as pathogen-resulting molecules that can mimic some or all the diseases symptoms, when they are introduced into plants at appropriate concentrations, induced by its producer. These toxins are usually divided into two groups: 1) non-host selective toxins (NHST) to which many plant species are sensitive, and 2) host-selective toxins (HSTs), where sensitivity is delimited to definite genotypes of the host (Otani et al., 1995). These are molecules ranging from low-molecular-weight metabolites to proteins. Host selective toxins are mostly active at concentrations ranging from 4.0 pM to 1 pM, and their degree of specificity (host selectivity) ranges from 100-fold to >106-fold (Waltan, 1996). Fungal genera including *Alternaria*, *Cochliobolus*, *Leptosphaeria*, *Venturia*, *Ascochyta* and *Pyrenophora* cause major diseases on cereals, legumes, apples and brassicas and these produce host selective toxins (Walton, 1996; Wolpert et al., 2002). The interactions between HSTs and their hosts are very specialized and opposite to classical gene-for-gene interaction. The sensitivity/susceptibility is usually conferred by a single dominant gene (Wolpert et al., 2002). Therefore, HST–host gene interactions are often seen as ‘‘inverse gene-for-gene’’ system.

P. tritici-repentis produces three host selective toxins that can cause the necrosis and/or chlorosis symptoms in susceptible wheat genotypes (Balance et al., 1989; Orolaza et al., 1995; Tomas and Bockus, 1987; Tuori et al., 1995). These three host-selective toxins are Ptr ToxA, Ptr ToxB, and Ptr ToxC (Effertz et al., 2002; Martinez et al., 2001; Orolaza et al., 1995). From the three toxins, Ptr ToxA (13.3-kDa in size) and Ptr ToxB (6.6 kDa) are proteintious in nature (Andrie and Ciuffetti, 2011; Kim et al., 2010; Martinez et al., 2004; Strelkov and Lamari, 2003) whereas, Ptr ToxC, which seems to be non-ionic, polar and low molecular weight in nature (Effertz et al., 2002). Another uncharacterized toxin, Ptr ToxD, have also been described (Wolpert et al., 2002). These three host-selective toxins are thought to be required by the fungus for pathogenesis (Lamari and Strelkove, 2010). Ptr ToxA and ToxB produce necrosis and chlorosis symptoms on wheat differential lines, Glenlea and 6B662, respectively whereas Ptr ToxC induces chlorosis on 6B365 (Effertz et al., 2002). Ptr ToxA is single copy gene and a small (13.2 kDa) secreted protein (Ballance et al., 1989; Tomas et al., 1990) and is present in approximately 80% of worldwide collection of *P. tritici-repentis* isolates (Friesen et al., 2006). Ptr ToxA is considered as a pathogenicity, virulence factor, and/or may not have any role in disease development depending on the wheat genotype (Ali et al., 2010; Ciuffetti et al., 1997; Friesen et al., 2003). Ciuffetti et al. (1997) transformed a non-pathogenic race 4 isolate with *Ptr ToxA* gene. They demonstrated that the isolate became pathogenic on Ptr ToxA sensitive wheat genotypes, and they considered it a pathogenicity factor. In contrast, another research group (Friesen et al., 2002) developed wheat mutants, (Ptr ToxA insensitive but tan spot susceptible) from the Ptr

ToxA-sensitive and tan spot-susceptible hard red spring wheat genotype ‘Kulm’. They inoculated both mutants and wild type plants with the fungus and found variation in disease progression between the mutants and wild-type plants over seven days’ period. The disease developed faster in wild-type plants as compared to the mutant plants and considered Ptr ToxA as a virulence factor.

Ptr ToxB is a 6.5 kDa protein (Martinez et al., 2001; Strelkov et al., 1999) produced by races 5, 6, 7, and 8 (Lamari et al., 2003). It causes chlorosis on toxin sensitive wheat genotypes and is encoded by multiple copies of the toxin gene (Martinez et al., 2001; Orolaza et al., 1995; Strelkov et al., 1999). The isolates with low and high *Ptr ToxB* gene numbers impact the pathogen virulence and hence varied in inducing the disease symptom. (Aboukhaddour et al., 2012; Andrie et al., 2008). A *Ptr ToxB* homolog *toxb*, was recognized in the non-pathogenic *P. tritici-repentis* race 4 and a pathogenic race 3, and in spite of its 86% resemblance to *Ptr ToxB*, *toxb* expresses an inactive protein (Martinez et al., 2004). Such a type of homologue was also observed in *Pyrenophora bromi*, the causal agent of brown leaf spot on smooth bromegrass, an alternative host of *P. tritici-repentis* (Andrie et al., 2008). The toxin affects the photosyntheic process by prompting chlorophyll degradation, generating oxidative bursts and reactive oxygen species accumulation which ultimately resulting in cell death (Kim et al., 2010; Strelkov et al., 1998) and impacting crop productivity.

Another host selective toxin “Ptr ToxC” is produced by *P. tritici-repentis* race 1, 3, 6, and 8 and induces extensive chlorosis in the toxin sensitive wheat genotypes (Effertz et al., 1998; Strelkov and Lamari, 2003). Although the toxin has not been completely

characterized yet, it has been purified partially through ion exchange and succeeding reverse face chromatography/gel filtration has been done. After reviewing the chemical nature of the toxin, it has been characterized as non-proteinaceous, non-ionic, low molecular weight (approximately 1.0 kDa) polar molecules (Effertz et al., 2002). The sensitivity of wheat genotypes to Ptr ToxC has been revealed to be independently inherited (Gamba et al., 1998; Gamba and Lamari, 1998). The sensitivity to Ptr ToxC is controlled by the gene *Tsc1* located on chromosome arm 1AS, which is liable for 26-64% of the variation in host resistance (Effertz et al., 2001). It is still unknown which gene is accountable for the synthesis of Ptr ToxC and its mode of action needs to be further studied (Lamari and Strelkov, 2010).

Virulence variation in *Pyrenophora tritici-repentis* population

Physiological variation in virulence and/or aggressiveness in *P. tritici-repentis* population have been reported using both quantitative (lesion number, lesion size, and/or necrotic leaf area) and qualitative (necrosis and/or chlorosis lesion type) rating scales (Da-Luz and Hosford, 1980; Krupinsky, 1987; Lamari and Bernier, 1989a; Misra and Singh, 1972; Schilder and Bergstrom, 1990). Misra and Singh (1972) recovered three single-spore isolates from wheat and tested them on 50 wheat cultivars. The cultivars varied in their reaction, based on lesion number and lesion size, to the tested isolates. Of 50 cultivars, 5 exhibited complete resistant against all the tested isolates. In another study, Da-Luz and Hosford (1980) recovered 40 single-spore *Pyrenophora trichostoma* isolates from wheat and evaluated them for their aggressiveness on five wheat cultivars. They grouped the isolates into twelve races based on lesion number and necrotic leaf

area developed on the susceptible cultivars. Similarly, 17 single spore isolates collected from Ontario (Canada); Maryland (USA); and New York (USA) were inoculated on wheat cultivars for studying their aggressiveness. The isolates differed in inducing lesion size and lesion number (Schilder and Bergstrom, 1990). Comparable results also obtained in other independent studies defining the physiological variation in *P. tritici-repentis* population (Hunger and Brown, 1987; Krupinsky, 1992a; Nasrellah, 1991). Though the isolates and wheat cultivars utilized in these studies showed variation in their reactions, they did not react differentially (races) e.g. a cultivar susceptible to one isolate, but not to the rest or an isolate virulent on one cultivar but avirulent on others. Since the race classification system based on lesion type in *P. tritici-repentis* has been established (Lamari et al., 1995), several studies have been conducted for detecting virulence variation in *P. tritici-repentis* populations (Ali and Buchneau, 1992; Ali and Francl, 2002; Ali and Francl, 2003; Ali et al., 2010; Benslimane et al., 2011; Engle et al., 2006; Friesen et al., 2005; Lamari and Bernier 1989b; Lamari et al., 1998 and 2005b; Singh et al., 2007). Ali and Francl (2003) characterized 270 isolates recovered from bread wheat, durum wheat, and non-cereal grasses for their race structure by inoculating them on the tan spot wheat differentials set. The isolates grouped into races 1, 2, 4, and 5 from wheat, and majority (98%) of the isolates from non-cereal grasses were designated as race 4 while the rest 20% were identified as race 1. The results of some other independent studies related to virulence variation suggest the involvement of some toxins in tan spot development other than the three already identified toxins (Ali et al., 2010, Andrei et al., 2007). Ali et al. (2010) characterized 61 isolates from wheat in

Arkansas for race identification. Nearly half of the isolates were grouped as race 1 based on their phenotypic reaction, but they lacked in *Ptr ToxA* gene. Similarly, in another study, two isolates SO3 and PT82 produced necrosis on Glenlea and chlororosis on 6B662, respectively like race 1 and race 5 but they were deficient in *Ptr ToxA* and *Ptr ToxB* genes. Benslimane et al. (2011) studied 55 *P. tritici-repentis* isolates collected from different wheat growing regions in Algeria to determine their race structure. They tested these isolates on both bread and durum wheat. In their study besides prevailing of commonly observed races they also found five isolates with a new virulence pattern that produced necrosis on durum wheat but were avirulent on bread wheat. They concluded that there might be new races evolving in that area. To know the fungal race structure in southern cone region of South America for the first time, Gamba et al. (2012) tested 273 *P. tritici-repentis* isolates collected from 87 wheat fields of three countries, Argentina, Brazil and Uruguay. In their evaluation, they classified 52.4% of the isolates as race 1, and rest were race 2. Their results showed the presence of less diversity in the pathogen from that region, and it might be using bread wheat tan spot susceptible cultivars to race 1 and race 2 on large area. Aboukhaddour et al. (2013) studied 45 isolates of *P. tritici-repentis* from Alberta, Canada by evaluating on six wheat differential lines for their races. According to their results, the most common race was race 1 (62%) followed by race 2 (36%) and race 3 (2%) in Alberta. Further all isolates designated as race 1 and 2 carried *Ptr ToxA* gene. One isolate identified as race 3 had *toxb*, a homologue of *ToxB*. Sarova et al. (2005) evaluated *P. tritici-repentis* race structure by testing 85 isolates from wheat and non-cereal grass species in Czech

Republic. Of 85 isolates, 50% were grouped as race 1, 2% race 2, and 5% classified as race 4. However, 42% of the isolates did not fall under the currently identified 8 races, indicating that prevalence of new races in the region and needs to be studied further. The results of these studies indicated that multiple races exist at various locations in many countries and that potentially additional virulence (toxins) or pathogenicity factors are present in the *P. tritici-repentis* responsible in tan spot development.

Host-Pathogen interaction

Numerous studies have been accomplished on wheat genetics for tan spot resistance/susceptibility and the qualitative and quantitative genes responsible for resistance were reported (Faris et al., 2013; Lamari and Bernier, 1989c; Lee and Gough, 1984; Nagle et al., 1982; Singh and Hughes, 2005; Singh et al., 2010). A single recessive gene *tsn1* located in chromosome 5BL that controls tan spot resistance against race 1 and 2 in common wheat and durum wheat was reported (Duguid and Babel, 2001; Faris et al., 1996; Gamba and Lamari, 1998; Stock et al., 1996). Wheat genotypes that lack in *Tsn1* gene sensitive to Ptr ToxA are significantly less susceptible to the most common pathogen races (Friesen et al., 2006). In relation to this scenario, Lamari et al. (2005a) found a link between *Tsn1* containing cultivars and susceptibility to tan spot among the Canadian wheat lines. They tested 86 wheat genotypes, which were developed during pre-and post-stem rust epidemics against *P. tritici-repentis* race 2, race 5 and with two host selective toxins Ptr ToxA and Ptr ToxB. They observed a positive correlation between sensitivity to toxin (both Ptr ToxA and Ptr ToxB) and susceptibility to the fungus (both race 2 and race 5) among the cultivars/genotypes.

On the contrary, another study conducted in Australia by Oliver et al. (2008) to find the relationship between *Tsn1*, responsible for Ptr ToxA sensitivity, containing cultivars and tan spot disease epidemics that occurred in Australia. Showed majority of the cultivars were insensitive for Ptr ToxA but were susceptible to the fungus and concluded an insignificant role of Ptr ToxA in tan spot epidemics. Similar results were also obtained in other independent studies (Faris and Friesen, 2005; Virdi et al., 2016). Virdi et al. (2016) developed two biparental wheat populations to study if Ptr ToxA has any association with tan susceptibility in durum, and they concluded that *Tsn1*-ToxA is not a significant factor for tan spot development but plays a major role in Stagonospora nodorum blotch, caused by *Stagonospora nodorum* containing *Ptr ToxA* gene, development in both common wheat and durum wheat. Their results explained this difference of host pathogen interaction between these two pathogens were due to the high level of Ptr ToxA expression in infected plants with *Tsn1* inoculated with *Parastagonspora nodorun* (*Stagonospora nodorum*) compared to *P. tritici-repentis*. So, from the above findings, it can be concluded that the *Tsn1*-Ptr ToxA interaction did not confer susceptible reaction in some specific genetic backgrounds, and this scenario can be explained by the broad spectrum of race non-specific resistance genes interplay in tan spot-wheat host-pathosystem. Considering this race non-specific resistance mechanism in the tan spot-wheat host-pathogen system, Kariyawasam et al. (2016) identified two major QTL in the two-parental population ‘Louise’ and ‘Penawawa’ responsible for tan spot development. Of these two QTLs, one is located on the chromosome arm 3BL and is associated with insensitivity to Ptr ToxA producing isolates,

and the other QTL is present on chromosome 1AS that is responsible for resistance to Ptr ToxC producing isolates. In this study, they observed that 'Penawawa' showed sensitivity to Ptr ToxA but was resistant to the toxin producer race 1. These findings inferred the race non-specific resistance in *P. tritici-repentis* host pathosystem exist.

The fungus *P. tritici-repentis* is a necrotrophic pathogen that produces three host selective toxins Ptr ToxA, ToxB and ToxC which are recognized by three independent corresponding sensitivity genes 'each gene for each toxin' in the host for compatible reaction following an inverse gene for gene model (Balance et al., 1989; Effertz et al., 2002; Orolaza et al., 1995; Tomas and Bockus, 1987). All genetic studies conducted so far to understand the genetic mechanism of tan spot development, *P. tritici-repentis* toxin and its producers spore inoculation mostly relied on the toxin sensitivity (Faris et al., 2013). Because the *P. tritici-repentis*-host gene interaction regulates race specificity in tan spot, they are reflected as race specific interactions. The host genes *Tsn1, Tsc1* and *Tsc2* responsible for Ptr ToxA, Ptr ToxB, and Ptr ToxC sensitivity have been mapped on wheat chromosome arms 5BL (Faris et al., 1996), 1AS (Effertz et al., 2001) and 2BS (Abeysekara et al., 2009; Friesen and Faris, 2004), respectively. Faris et al. (2010) cloned the *Tsn1* gene and designated it as *R* gene which encoded protein kinase, nucleotide binding domains and Lucien-rich repeat domains. However, in addition to those sensitivity genes there are some other qualitative genes that were identified for tan spot resistance (Singh et al., 2006, 2008; Tadesse et al., 2006a; Tadesse et al., 2006b) These findings conferred the presence of race specific and

race non-specific resistance genes to tan spot present in the host depending on the wheat genotype.

Rationale of the study

Wheat (*Triticum aestivum* L.) is the major cereal crop grown worldwide for food, feed and other products. Wheat grains are highly nutritive as they are rich in energy, carbohydrates, dietary fiber, fat, protein, vitamins and various minerals and consumed in various forms across the globe (USDA-National Nutrient Database for Standard Reference, Release 19, 2006). In the year 2016, 27.31 billion bushels of wheat were produced worldwide, whereas the US alone produced 2.3 billion bushels hence ranked fourth after China, European Union and India in world-wide wheat production (USDA-FAS, 2016).

In South Dakota, hard red winter, and hard red spring wheat are primarily produced with the production of 103.40 million bushels on about 2.1 million acres in the year 2016 (NASS-USDA-2016). Wheat productivity can be impacted drastically (20-40%) by pests and diseases (FAO, 2012). Leaf spot diseases (tan spot, stagnospora nodorum blotch, and spot blotch) can cause significant yield losses (3-53%; 30%; >50% respectively) in wheat production (Shabeer and Bockus, 1988; Villareal et al., 1995; Bhathal et al., 2003). Of these three leaf spot diseases, tan spot is the most prevalent and the first disease appearing during the wheat-growing season on seedlings and remains present until wheat matures in the Dakotas (Friskop and Liu, 2016; Byamukama, 2013). The disease can cause yield loss up to 53% under favourable

environment for disease development in the US Great Plains (Shabeer and Bockus, 1988). In South Dakota, tan spot can impact crop productivity from 5-29% (Buchneau et al., 1983). The disease can be primarily managed through deployment of tan spot resistant cultivars and fungicide application (DeWolf et al., 1998). So far, most of the commercial wheat cultivars cultivated in the Dakotas are susceptible to moderately susceptible to tan spot with few exceptions. Eight races have been identified so far in tan spot causing fungus *Pyrenophora tritici-repentis* based on their ability to produce two distinct necrosis and chlorosis symptoms and three host selective toxins associated with the two symptoms have been identified (Lamari et al., 2003). Of these eight currently identified races, races 1 through 5 have been observed in the USA (Abdullah et al., 2016; Ali and Francl, 2003; Ali et al., 2010; Engle et al., 2006). *Pyrenophora tritici-repentis* also has a wide host range on cereal and non-cereal grasses (Ali and Francl, 2003; Krupinsky, 1982, 1992a; Sprague, 1950). Alternate hosts can play a significant role in pathogen diversity and disease epidemic (Burdon 1993); especially when they are cultivated in the vicinity of the economical host. In contrast, they can also be a part of the solution by contributing resistance genes to pests and diseases (Dinoor, 1974). Historically, tan spot was observed on wheat from various parts of the world in the 1930's, and 1940's (DeWolf et al., 1998; Hosford 1982), but it was not considered a major threat to wheat production until the 1970's. The dramatic increase in tan spot spread and severity worldwide in the 1970's and onward is anecdotally considered to be due to: 1) deployment of stem rust resistance cultivars to fight against stem rust epidemics on the 1930's, and 1950's; 2) increase in the pathogen virulence due to

acquiring *Ptr ToxA* gene from *Stagnospora nodorum*; and change in tillage practices from conventional to zero or minimal that help the residue borne pathogen like *P. tritici-repentis* from one growing season to the next hence increase in inoculum level.

Knowledge of genetic variation in the pathogen virulence and resistance in the host plant is crucial in the development of effective and durable disease management strategies especially breeding for durable disease resistant cultivars. Variability in virulence and aggressiveness has been reported in the *P. tritici-repentis* isolates recovered from wheat, barley and various alternative hosts such as Altai wild rye, Basin wild rye, and Russian wild rye (Aboukhaddour et al., 2013; Ali and Francl, 2003; Ali et al., 2010; Andrie et al., 2007; Benslimane et al., 2011; Gamba et al., 2012, Krupinsky, 1992a; Lamari et al., 2003; Lamari and Bernier 1989a). Rye is one of the important cereal crops worldwide primarily grown as forage for livestock, cover crop for green manure, and food products like bread (Bushuk, 2001). Moreover, rye has a great significance in the modern wheat improvement being a contributor of resistance genes for fighting against leaf rust, stem rust, stripe rust, powdery mildew, BYDV and insect resistance to Hessian fly, Russian wheat aphids, and green bug (Rabinovich 1998; Saulescu et al., 2011).

In the Northern Great Plains, rye is grown as a green manure and a rotational crop and is mostly planted adjacent to wheat fields. This can potentially play a role in *P. tritici-repentis* virulence variation and evolution; harbor completely different pathogen population; and/or serve as an additional source of inoculum for tan spot development in wheat. Previously, barley and oats have been tested against tan spot reaction and reported to be resistant (Hosford, 1971b; Ali and Francl, 2001). Further, the fungal

population prevalent on barley in North Dakota was identified as race 1, and the study concluded an insignificant role of barley in tan spot disease epidemiology and pathogen diversity (Ali and Francl, 2001). However, prevalence of *P. tritici-repentis* population on rye has not been studied. Further, diversity to tan spot reaction in rye has not been explored in the region.

The fungal population from Czech Republic, Poland, Germany and Russia has been analyzed for virulence variation (Leisova-Svobodova et al., 2010; Mikhalova et al., 2014; Mironenko et al., 2015; Sarova et al., 2005; Zamorski and Schollenberger, 1994), however, information on the *P. tritici-repentis* virulence variation prevalent on wheat from Latvia, Lithuania neighboring states of Russia and Romaina neighboring state to Poland and Czech Republic is not available. Moreover, there is little information available on the genetic variability in *P. tritici-repentis* population in South Dakota (Ali and Buchenau, 1992; Ali and Francl, 2003). So, the information of *P. tritici-repentis* virulence diversity from three east European countries will fill the gap in the region from Russia to Poland as well as two wheat growing regions of Northern Great Plains, of South Dakota and Nebraska.

Research Objectives

The objectives of our study were 1) determine the correlation between tan spot increase in the 1970's and deployment of stem rust resistance cultivars and change in the pathogen virulence due acquisition of *Ptr ToxA* gene from *Stagnospora nodorum;* 2) study *P. tritici-repentis* diversity in wheat in South Dakota and Baltic states; and 3)

determine role of rye in *P. tritici-repentis* diversity in South Dakota and reaction of global collection of rye germplasm to tan spot.

LITERATURE CITED

Abdullah, S., Byamukama, E., Langham, M. G. H., and Ali, S. 2016. First report of *Pyrenophora tritici-repentis* race 5 from South Dakota. *Plant Dis.* 100:2322.

Abeysekara N. S., Friesen, T. L., Keller, B., and Faris, J. D. 2009. Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. *Theor Appl Genet.* 120:117–26.

Aboukhaddour, R., Kim, Y. M., and Strelkov, S. E. 2012. RNA-mediated gene silencing of ToxB in *Pyrenophora tritici-repentis*. *Mol. Plant Path.* 13:318-326.

Aboukhaddour, R., Turkington, T. K., and Strelkov, S. E. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada, *Can. J. Plant Path.* 35:256-268.

Aboukhaddour, R., and Strelkov, S. E. 2016. Exploring de novo specificity: The *Pyrenophora tritici-repentis*–barley interaction. *Plant Path*. 65:1347–1357.

Alfano J. R., and Collmer A. 1996. Bacterial Pathogens in Plants: Life up against the Wall. Plant Cell. 88:1683–1698.

Ali, S., and Buchenau, G. W. 1992. Incidence of toxin producing pathotypes of *P. tritici-repentis* in South Dakota. *Phytopathology* 82:1159 (Abstract).

Ali, S., and Francl, L. J. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. *Plant Dis.* 85:580-584.

Ali, S., and Francl, L. J. 2002. Race structure of *Pyrenophora tritici-repentis* isolates obtained from wheat in South America. *Plant Prot. Sci.* (special issue) 38:302-304.

Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses. *Plant Dis.* 87: 418-422.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Dis.* 94:229–235.

Amaike, S., Ozga, J. A., Basu U., and Strelkov, S. E. 2008. Quantifiction of *ToxB* gene expression and formation of appressoria by isolates of *Pyrenophora tritici-repentis* differing in pathogenicity. *Plant Path.* 57:623-633.

Andersen, H. 1955. Species of *Helminthosporium* on cereals and grasses in Denmark. *Friesia* 5:80-89.

Andrie, R. M., Martinez, J. P., Ciuffetti, L. M., 2005. Development of ToxA and ToxB promoter-driven fluorescent protein expression vectors for use in filamentous ascomycetes. *Mycologia* 97:1152–1161.

Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007. A combination of phenotipic and genotypic characterization *Pyrenophora tritici-repentis* race identification. *Phytopathology* 97:694-701.

Andrie, R. M., Schoch, C. L., Hedges, R., Spatafora, J. W., and Ciuffetti, L. M. 2008. Homologs of ToxB, a host-selective toxin gene from *Pyrenophora tritici-repentis*, are present in the genome of sister-species *Pyrenophora bromi* and other members of the Ascomycota. *Fungal Genet. Biol.* 45:363-377.

Andrie, R. M, and Ciuffetti, L. M. 2011. *Pyrenophora bromi*, causal agent of brown spot of brome grass, expresses a gene encoding a protein with homology and similar activity to Ptr ToxB, a host-selective toxin of wheat. *Mol. Plant Microbe Interact.* 24:359–367.

Ballance, G. M., Lamari, L., and Bernier, C. C. 1989. Purification of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. *Physiol. Mol. Plant Pathol.* 35:203-213.

Barrus, M. F., and Johnson, A. G. 1942. Yellow - spot disease of wheat in the New York State. *Helminthosporium tritici-vulgaris* on wheat in Maryland. *Plant Dis. Rep.* 26:246.

Benslimane, H., Lamari, L., Benbelkacem, A., Sayoud, R., and Bouznad, Z. 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identification of a new virulence type. *Phytopathol. Mediterr.* 50:203−211.

Bhathal, J. S., Loughman, R. Speijers, J. 2003. Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and *Septoria nodorum* blotch. *Euro. J. Plant Path*. 109: 435–443.

Buchenau, G. W., Smolik, J. D., Ferguson, M. W., and Cholick, F. A. 1983. Wheat diseases in South Dakota. Ann. Wheat Newsletter 29:147.

Burdon, J. J. 1993. The structure of pathogen populations in natural plant communities. *Annu. Rev. Phytopath.* 31:305-323.

Bushuk, W. 2001. RYE: Production, Chemistry, and Technology, Second Edition AACC, St Paul, MN.

Byamukama, E. 2013. Leaf spot diseases beginning to develop in winter wheat. igrow. SDSU Extension 6/6/2013.

Ciuffetti, L. M., Tuori, R. P., and Gaventa, J. M. 1997. A single gene encodes a selective toxin causal to the development of tan spot on wheat. *The Plant Cell* 9:135-144.

Ciuffetti, L. M., Francl, L. J., Ballance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., and Rasmussen, J. B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction, *Can. J. Plant Path.* 20:421-424.

Cooners, L. L. 1937.17th Annu. Rep. Can. *Plant Dis.* Surv. p. 5.

Conners, I. L. 1939. Nineteenth annual report of the Canadian Plant Disease Survey 19: 12-14.

Da Luz, W. C., and R. M. Hosford, Jr. 1980. Twelve *Pyrenophora trichostoina* races for virulence to wheat in the central plains of North America. *Phytopathology* 70:1193-1196.

Dennis, R. W.G., and Wakefield, E. M. 1946. New or interesting Bitrish fungi. *Trans Brit. Mycol Soc.* 29:141-166.

De Wolf, E. D., Effertz, R. J., Ali, S., and Francl, L. J. 1998. Vistas of tan spot research. *Can. J. Plant Pathol.* 20:349-370.

Dickson, J. G. 1956. Diseases of Field Crops. Mc Graw Hill Book Co. New york, 517 pp.

Diedicke, H. 1902. Ueber den *Zussammenhang Zwischen Pleospora* - und *Helminthosporium* - Arten. Centrablatt fur Bakteriologie und Parasitenkunde Jena, Abt. 9:317-329.

Diedicke, H. 1904. Ueber den *Zusammenhang zwischen Pleospora* und *Helminthosporium arten*. Zeitschirft fur Pflanzenkrankheiten 11:317-329.

Dinoor, A. 1974. Role of wild and cultivated plants in the epidemiology of plant diseases in Israel. *Annu. Rev. Phytopathol.* 12:413-436.

Dreschler, C. 1923. Some graminicolous species of *Helminthosporium* :1. *J. Agril. Res.* 24:641-740.

Duff, A. D. S. 1954. A new disease of wheat in Kenya caused by a specie of *Pyrenophora*. *The East African Agri. J.* 225-229.

Duguid, S. D., and Brûlé-Babel, A. L. 2001. Inheritance and interaction of spring wheat (*Triticum aestivum* L.) resistance to race 2 and race 3 *of Pyrenophora tritici-repentis* (Died.) Drechs. *Can. J. Plant Sci.* 81:527-533.

Dushnicky, L. G., MacGregor, A. W., Summer, M. J., and Ballance, G. M. 1996. Penetration and infection of susceptible and resistant wheat cultivars by a necrosis toxin-producing isolate of *Pyrenophora tritici-repentis*. *Can. J. Plant Path.* 18:392-402.

Dushnicky, L. G., Ballance, G. M., Sumner, M. J., and MacGregor, A. W. 1998a. Detection of infection and host responses in susceptible and resistant wheat cultivars to a toxin producing isolate of *Pyrenophora tritici-repentis*. *Can. J. Plant Path.* 20:19-27.

Dushnicky, L. G., Ballance, G. M., Sumner, M. J., and MacGregor, A. W. 1998b. The role of lignification as a resistance mechanism in wheat to a toxin-producing isolate of *Pyrenophora tritici-repentis. Can. J. Plant Path.* 20:35-47.

Effertz, R. J., Anderson, J. A., and Francl, L. J. 1998. QTLs associated with resistance to chlorosis induction by *Pyrenophora tritici-repentis* in adult wheat. *Can. J. Plant Pathol.* 20:438–439.

Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. *Phytopathology* 91:572- 578.

Effertz, R. J., Meinhardt S. W., Anderson J. A., Jordahl J. G., Francl L. J. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. *Phytopathology* 92:527–533.

Ellis, M. B., and Waller, J. M. 1976. *Pyrenophora tritici-repentis* (conidial state: *Drechslera tritici-repentis*). Commonwealth Mycol. Inst. Description Pathogenic Fungi Bacteria No. 494.

Engle, J. S., Madden, L. V., and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology*. 96:1355-1362.

FAO, 2012. Monitoring and surveillance of cereals pests, diseases and weeds. Sub-regional Office of FAO for Central Asia (FAO-SEC), December 2012 p: 07.

Faris, J. D., and Friesen, T. L. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. *Theor. Appl. Genet.* 111:386–392.

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology.* 86:459- 463.

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy L. et al., 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci.* USA 107:13544–13549.

Faris, J. D., Liu, Z., and Xu, S. S. 2013. Genetics of tan spot resistance in wheat. *Theor. App. Genet*:1–21.

Farr, D. F., Bills, G. F., Chamuris, G. P. and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. APS Press, St Paul MN. 1252 pp.

Fernandez, M. R., Depauw, R. M., and Clarke, J. M. 2001. Reaction of common and durum wheat cultivar to infection of kernels by *Pyrenophora tritici-repentis*. *Can. J. Plant path.* 23:158-162.

Francl, L. J. 1997. Local and mesodistance dispersal of *Pyrenophora tritici-repentis* conidia. *Can. J. Plant Pathol.* 19:247-255.

Friesen, T. L., Rassmussen. J. B., Ali, S., Kwon, C. Y., and Francl, L. J. 2002. Reaction of Ptr ToxA insensitive wheat mutants to *Pyrenophora tritici-repentis* race 1. *Phytopathology* 92:38-42.

Friesen, T. L., Ali, S. Kianian, S. Francl, L. J. and Rasmussen, J. B. 2003. Role of host sensitivity to Ptr ToxA in the development of tan spot. *Phytopathology* 93:397-401.

Friesen, T. L., and Faris, J. D. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. *Theor. Appl. Gene.t* 109: 464–471.

Friesen, T. L., Ali, S., Kleim, K. K. & Rasmussen, J. B. 2005. Population genetic analysis of a global collection of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Phytopathology* 95:1144-1150.

Friesen, T. L., E. H. Stucken Brock, Liu, Z. H., Meinhardt, S., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetic* 38:953-956.

Friskop, A., and Liu, Z. 2016. Fungal Leaf Spot Diseases of Wheat: Tan spot, *Septoria*/*Stagonospora nodorum* blotch and *Septoria tritici* blotch. PP1449, NDSU Extension Bull. NDSU, Fargo, ND58107

Gamba, F. M., and Lamari, L. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). *Can. J. Plant Path.* 20:408–414.

Gamba, F. M., Lamari, L., and Brule-Babel, A. L. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. *Can. J. Plant Path.* 20:401-407.

Gamba, F. M., Strelkov, S. E., and Lamari L. 2012. Virulence of *Pyrenophora tritici-repentis* in the southern cone region of south America. *Can. J. Plant Path.* 34:545–550.

Hosford, R. M. 1971a. Wheat leaf blight and blotch losses and control. Farm Research p:5-8.

Hosford, R. M. 1971b. A form of *Pyrenophora trichstoma* patho¬genic to wheat and other grasses. *Phytopathology* 61:28-32.

Hosford, R. M. 1972. Propagules of *Pyrenophora trichostoma*. *Phytopathology.* 62:627-629.

Hosford, R. M. 1974. Epidemiology and control of leaf spots of wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f.sp. triticea. Abstr. No. 386. In Abstracts of papers, 66th Annu. Meet. *Am. Phytopathol. Soc.* 40th Session *Can. Phytopathol. Soc.,* Vancouver, B.C., Canada, Aug. 11115; 1974.

Hosford, R. M. Jr. 1982. Tan spot - Developing knowledge 1902 - 1981. In Tan Spot of Wheat and Related Diseases Workshop. July. 1981. Fargo, N.D. Edited by Hosford, R. M.

Jr. North Dakota Agricultural Experimental Station, North Dakota State University, Fargo, N.D. pp:1-24.

Hosford, R. M. Jr., Larez, C. R., and Hammond, J. J. 1987. Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheat of differing resistance. *Phytopathology* 77:1021-1027.

Howard, R. J., and Morrall, R. A. A. 1975. The epidemiology of leaf spot disease in a native prairie. I. The progression of disease with time. *Can. J. Bot.* 53:1040-1050.

Hunger, R. M., and Brown, D. A. 1987. Colony color, growth, sporulation, fungicide sensivity and pathogenicity of *Pyrenophora tritici-repentis*. *Plant Dis.* 71:907-910.

Kariyawasam, G. K., Carter, A. H., Jack B., Rasmussen, J. B., Faris, J., Xu, S. S., Mergoum, M. and Liu, Z. 2016. Genetic relationships between race-nonspecific and race-specific interactions in the wheat–*Pyrenophora tritici-repentis* pathosystem. *Theor. Appl. Genet.* 129:897–908.

Kim, Y. M, Bouras, N, Kav, N. N, and Strelkov, S. E. 2010. Inhibition of photosynthesis and modification of the wheat leaf proteome by Ptr ToxB: a host-specific toxin from the fungal pathogen *Pyrenophora tritici-repentis*. *Proteomics* 10:2911–2926.

Kohli, M., Mehta, Y., and Ackermann, M. 1992. Spread of tan spot in the Southern Cone Region of South America. In L. Francl, J. Krupinsky, & M. Mc Mullen (Eds.) Advances in Tan Spot. Proceedings of the Second International Tan Spot Workshop (pp. 86–90). Fargo, ND: North Dakota State University.

Krupinsky, J. M. 1982. Observation of the host range of isolates of *Pyrenophora trichostoma*. *Can. J. Plant Pathol.* 4:42-46.

Krupinsky, J. M. 1987. Pathogenicity on wheat of *Pyrenophora tritici-repentis* isolated from *Bromus inermis*. *Phytopathology* 77:760-765.

Krupinsky, J. M. 1992a. Grass hosts of *Pyrenophora tritici repentis*. *Plant Dis.* 76:92–95.

Krupinsky, J. M. 1992b. Aggressiveness of isolates of *Pyrenophora tritici-repentis* obtained from wheat in the northern Great Plains. *Plant Dis.* 76:87-91.

Lamari, L., and Bernier, C. C. 1989a. Evaluation of wheat for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. *Can. J. Plant Pathol.* 11:49-56.

Lamari, L. and Bernier, C. C. 1989b. Virulence of isolates of *Pyrenophora tritici-repentis* on eleven wheat cultivars and cytology of the differential host reaction. *Can J. Plant Path.* 11: 284-290.

Lamari, L. and Bernier, C. C. 1989c. Toxin of *Pyrenophora tritici-repentis*: Host-Specificity, Significance in Disease, and Inheritance of Host Reaction. *Phytopathology* 79:740-744

Lamari, L., Sayoud, R., Boulif, M., & Bernier, C.C. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. *Can. J. Plant Pathol.* 17:312–318.

Lamari, L., Gilbert, J., and Tekauz, A. 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western *Canada. Can. J. Plant Pathol.* 20:396–400.

Lamari, L., Srelkove, S. E., Yahyaoui. A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one to one relationship in tan spot of wheat. *Phytopathology* 93:391-396.

Lamari, L., McCallum, B. D., and Depauw, R. M. 2005a. Forensic pathology of Canadian bread wheat: The case of tan spot. *Phytopathology* 95:144-152.

Lamari L., Strelkov S. E., Yahyaoui A., Amedov M., Saidov M., et al., 2005b. Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road. *Can. J. Plant Pathol.* 27:383–388.

Lamari, L., and Strelkov, S. E. 2010. The wheat/*Pyrenophora tritici repentis* interaction: progress towards an understanding of tan spot disease. *Can. J. Plant Pathol.* 32:4–10.

Larez, C. R., Hosford, R. M., and Freeman, T. P. 1986. Infection of wheat and oats by *Pyrenophora tritici-repentis* and initial characterization of resistance. *Phytopathology* 76:931-938.

Lee, T. S., and Gough, F. J. 1984. Inheritance of *Septoria leaf blotch* (*S. tritici*) and *Pyrenophora* tan spot (*P. tritici-repentis* resistance in *Triticum aestivum* cv. Carifen 12. *Plant Dis.* 68:848-851.

Leisova-Svobodova, L., Hanzalova, A., and Kucera, L. 2010. Expansion and variability of the *Ptr ToxA* gene in populations of *Pyrenophora tritici-repentis* and *Pyrenophora teres. J. of Plant Path.* 92:729-735.

Lepoint, P., Renard, M. E., Legreve, A., Duveiller, E., and Maraite, H. 2010. Genetic Diversity of the Mating Type and Toxin Production Genes in *Pyrenophora tritici-repentis*. *Phytopathology* 100:474-483.

Manisha, S. 2016. Germplasm enhancement for yellow spot resistance in wheat. Dept. of Agric. and Food, WA. https://agric.wa.gov.au/n/2813

Martinez, J. P., Ottum, S. A., Ali, S., Francl, L. J., Ciuffetti, L. M. 2001. Characterization of the ToxB gene from *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.*14:675-677.

Martinez, J. P., Oesch, N. W., and Ciuffetti, L. M. 2004. Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. *Mol. Plant-Microbe Interact.* 17:467-474.

Mikhalova, L. A., Mironenko, N. V. and Kovalenko, N. M. 2014. Populations of *Pyrenophora tritici-repentis* in the North Caucasus and North-West Russia: The racial composition and dynamics of virulence. *Mikol. Fitopatol.* 48:393–400.

Mironenko, N. V., Baranova, O. A., Kovalenko, N. M. and Mikhalova, L. A. 2015. Frequency of ToxA gene in North Caucasian and North-West Russian populations of *Pyrenophora tritici-repentis*. *Mikol. Fitopatol.* 49:325–329.

Misra, A. P., and Singh, R. A. 1972 Pathogenic differences amongst three isolates of *Helminthosporium tritici-repentis* and the performance of wheat varieties against them. Indian *Phytopathology* 25:350–353.

Mitra, M. 1934. A leaf spot disease of wheat caused by *Helminthosporium tritici-repentis* Died. *Ind. J. Agric. Sci.* 4:692-700.

Nagle, B. J., Frohberg, R. C., and Hosford, R. M., Jr. 1982. Inheritance of resistance to tan spot of wheat. Pages 40-45 in: Tan Spot of Wheat and Related Disease Workshop. R. M. Hosford, Jr., ed. North Dakota State University, Fargo.

NASS-USDA 2016. South Dakota small grain acreage and production (http://usda.mannlib.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-30-2016.pdf).

Nisikado, Y. 1928. Preliminary notes on yellow leaf spot diseases of wheat caused by *Helminthosporium tritici-vulgaris* Nisikado. *Inst. Agric. Biol.* 4:103-109.

Nsarellah, N. 1991. Evaluation of tan spot on wheat in North Dakota and Morocco. Ph.D. Diss., North Dakota State University. 93 pp.

Oliver, R. P., Lord, M., Rybak, K., Faris, J. D., and Solomon, P. S. 2008. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin sensitive cultivars. *Phytopathology* 98:488-491.

Orolaza, N. P., Lamari, L., and Balance, G. M. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. *Phytopathology* 85:1282-1287.

Otani, H., Kohmoto, K., and Kodama, M. 1995. *Alternaria* toxins and their effects on host plants. *Can. J. Bot.* 73:S453-S458.

Rabinovich, S. V. 1998. Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L. *Euphytica* 100:323–340.

Rees, R. G., and Platz, G. J. 1979. The occurrence and control of yellow spot of wheat in northeastern Australia. *Aust. J. Exp. Agric. Anim. Husb.* 19:369-72.

Rees, R. G., and Platz, G. J. 1983. Effect of yellow leaf spot on wheat: Comparison of epidemics at different stages of crop development. *Aust. J. Agric. Res.* 34:39-46.

Sarova, J., Hanzalova, A., and Bartos, P. 2005. Races of *Pyrenophora tritici-repentis* in the Czech Republic. *Acta Agrobotanica* 58:73-78.

Saulescu, N. N., Ittu, G., Ciuca, M., Ittu, M., Serban, G., and Mustafa, P. 2011. Transferring useful rye genes to wheat, using triticale as a bridge. *Czech J. Genet. Plant Breed.* 47(Special Issue):56–62.

Schilder, A. M. C., and Bergstrom, G. C. 1990. Variation in virulence within the population of *Pyrenophora tritici-repentis* in NewYork. *Phytopathology* 80:84-90.

Schilder, A. M. C., and Bergstrom, G. C. 1993. Tan spot. In S.B. Mathur & B.M. Cunfer, eds. *Seedborne Diseases and Seed Health Testing of Wheat*, p. 113-122. Copenhagen, Denmark, Jordburgsforlaget.

Shabeer, A., and Bockus, A. A. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Dis.* 72:599-602.

Shaw, D. E., and Valder, P. G. 1953. A study of microflora of wheat grains in New South Wales. Proc. Linn. Soc. N. S. W. 77:307-322.

Shoemaker, R. A. 1962. Drechslera Ito. *Can J. Bot.* 40:809-836.

Singh, P. K., and Hughes, G. R. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, race 1 and 2, in spring and winter wheat genotypes. *Phytopathology* 95:172–177.

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E., and Hughes, G. R. 2006. Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. *Phytopathology* 96:885–889.

Singh, P. K., Mergoum, M., & Hughes, G. R. 2007. Variation in virulence to wheat in *Pyrenophora tritici-repentis* population from Saskatchewan, Canada, from 2000 to 2002. *Can. J. Plant Pathol*. 29, 166–171.

Singh, P. K., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T.B., Kianian, S. F., Elias, E.M., and Hughes, G. R. 2008. Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. *Mol. Breed.* 21:293–304.

Singh, P. K., Mergoum, M., Adhikari, T. B., Shah, T., Ghavami, F., and Kianian, S. F. 2010. Genetic and molecular analysis of wheat tan spot resistance effective against *Pyrenophora tritici-repentis* races 2 and 5. *Mol. Breed.* 25:369–379.

Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press Co., New York. 538 pp.

Strelkov, S. E., Lamari, L., and Ballance, G. M. 1998. Induced chlorophyll degradation by a chlorosis toxin from *Pyrenophora tritici-repentis*. *Can. J. Plant Path.* 20:428-435.

Strelkov, S. E., Lamari, L., and Ballance, G. M. 1999. Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. *Mol. Plant–Microbe* Interact. 12:728–732.

Strelkov, S. E., Lamari, L., Sayoud, R., and Smith, R. B. 2002. Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. *Can. J. Plant Path.* 24: 29-35.

Strelkov, S.E., and Lamari, L. 2003. Host-parasite interaction in tan spot (*Pyrenophora tritici-repentis*) of wheat. *Can. J. Plant Path*. 25:339-349.

Stock, W. S., Brule-Babel, A. L., and Penner, G. A. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of *Triticum aestivum* cv. Chinese Spring. *Genome* 39:598-604.

Tadesse W, Hsam, S. L., Wenzel, G., Zeller and F. J. 2006a. Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L. × *Aegilops tauschii* Coss.). *Crop Sci.* 46:1212–1217.

Tadesse W, Hsam, S. L. K., and Zeller, F. J. 2006b. Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. *Plant Breed.* 125:318–322.

Tekauz, A. 1976. Distribution, severity and relative importance of leaf spot diseases wheat in Western Canada en 1974. *Can. Plant. Dis. Surv.* 56:36-40.

Tomas, A., and Bockus, W. W. 1987. Cultivar specific toxicity of culture filtrate of *Pyrenophora tritici-repentis*. *Phytopathology* 77:1337-1366.

Tomas, A., Feng, G. H., Bockus, W. W., and Leach, J. E. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Mol. *Plant-Microbe Interact*. 3:221-224.

Tuori, R. P., Wolpert, T. J., and Ciuffetti, L. M. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis.* Mol. *Plant-Microbe Interact*. 8:41-48.

USDA, 2006. National Nutrient Database for Standard Reference, Release 19. Nutrient Data Laboratory Home Page, /ba/bhnrc/ndl

USDA-FAS 2016. Overview-wheat world market and trade (https://apps.fas.usda.gov/psdonline/circulars/grain-wheat.pdf)

Villareal, R. L., Mujeeb Kazi, A., Gilchrist, L. I. and Del Toro, E. 1995 Yield loss to spot blotch in spring bread wheat in warm non-traditional wheat production areas. *Plant Dis.* 79:893–897.

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and *Septoria nodorum* blotch. *G3: Genes|Genomes|Genetics* 6:4139-4150.

Walton, J. D. 1996. Host-Selective Toxins: Agents of Compatibility Jonathan. *The Plant Cell* 8:1723-1733.

Wolf, P. F. J., and Hoffmann, G. M., 1993. Zur Biologie von *Drechslera tritici-repentis* (Died.) Shoem. (telomorph *Pyrenophora tritici-repentis* (Died.) Drechsler), dem Erreger einer Blattfleckenkrankheit an Weizen. Z. Pfl.krankh. *Pflschutz* 100:33—48.

Wolpert, T. J., Dunkle, L. D., Ciuffetti, L. M. 2002. Host-selective toxins and avirulence determinants: what's in a name? *Annu. Rev. Phytopathol.* 40:251–85.

Zamorski, C., and Schollenberger, M. 1994 The occurence of tan spot of wheat and triticale in Poland. - *Genetica Polonica* 35B:375-378.

Zeller, F. J., and Hsam, S. L. K. 1983. Broadening the genetic variability of cultivated wheat by utilizing rye chromatin. In: Proc. 6th Int. Wheat Genetic Symposium. Kyoto, 161–173.

CHAPTER 2

TITLE: INSIGHTS INTO TAN SPOT AND STEM RUST RESISTANCE AND SUSCEPTIBILITY BY STUDYING THE PRE-GREEN REVOLUTION GLOBAL COLLECTION OF WHEAT

Abdullah, S., Sehgal, K. S., Jin, Y., Turnipseed, B., and Ali, S. 2017. Insights into tan spot and stem rust resistance and susceptibility by studying the pre- green revolution global collection of wheat. *Plant Pathol. J.* 33: 125-132.

ABSTRACT

Tan spot (TS), caused by the fungus *Pyrenophora tritici-repentis* (Died) Drechs, is an important foliar disease of wheat and has become a threat to world wheat production since the 1970's. In this study, a globally diverse pre-1940's collection of 247 wheat genotypes was evaluated against Ptr ToxA, *P. tritici-repentis* race 1, and stem rust to determine if: i) acquisition of Ptr ToxA by the *P. tritci-repentis* from *Stagonospora nodorum* led to increased pathogen virulence or ii) incorporation of TS susceptibility during development stem rust resistant cultivars led to an increase in TS epidemics globally. Most genotypes were susceptible to stem rust, however, a range of reactions to TS and Ptr ToxA were observed. Four combinations of disease-toxin reactions were observed among the genotypes: TS susceptible-Ptr ToxA sensitive, TS susceptible-Ptr ToxA insensitive; TS resistant-Ptr ToxA insensitive, and TS resistant-Ptr ToxA toxin sensitive. A weak correlation (r = 0.14 for bread wheat and -0.082 for durum) was observed between stem rust susceptibility and TS resistance. Even though there were no TS reported epidemics in the pre-1940's, TS sensitive genotypes were widely grown in that period, suggesting that Ptr ToxA may not be an important factor responsible for enhanced prevalence of tan spot.

Keywords: wheat, yellow spot, host-selective toxin, foliar disease, *Puccinia graminis* f. sp. *tritici*

INTRODUCTION

Introduction of a new pathogen/race or change in virulence in an already existing pathogen population, deployment of a high yielding but susceptible cultivars on a large area, and changes in cultural practices that aid pathogen survival could change the status of a minor disease to a major one and create a disease epidemic in a region. These scenarios are probably responsible for an increase in tan spot in major wheat growing regions since the 1970's. Tan spot (yellow spot), caused by the fungus *Pyrenophora tritici-repentis* (Died) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoem.), is an important foliar disease of wheat in major wheat growing countries, especially in the US Northern Great Plains (Hosford, 1982). In addition to wheat, the pathogen can survive and infect many non-cereal grasses (Ali and Francl, 2003; Hosford, 1982; Krupinsky, 1992; Sprauge, 1950). *Pyrenophora tritici-repentis* (*Ptr*) can survive on infested crop residue from one growing season to the next, can also be spread through infected seed, and can thus be dispersed over a long distance from one country to another (Hosford, 1982; Schilder and Bergstrom, 1995). The fungus produces lens-shaped necrotic lesions encircled by a chlorotic halo with a pinhead size black spot in the leaf center of tan spot susceptible wheat cultivars. Losses due to reduction of leaf photosynthetic area caused by tan spot have been reported to be up to 50%, depending upon the cultivar, pathogen virulence, growth stage, and favorable environment for the disease development (Rees and Platz, 1983; Shabeer and Bockus, 1988). The fungus was initially reported in the 1850's from Germany on *Agropyron repens* (Diedicke, 1902), however, it was first reported causing leaf spot on wheat in Japan (Nisikado, 1928), and

soon after in Canada (Conners, 1932) and India (Mitra, 1934). Thereafter, the disease was observed in Australia, USA, and many other wheat-growing countries (Barrus, 1942; DeWolf et al., 1998; Rees and Platz, 1983). Though the disease was observed on wheat in the 1920's, it was not considered a major disease or a potential threat to wheat production until the 1970's (DeWolf et al., 1998; Hosford, 1982; Moreno et al., 2012; Rees and Platz, 1983). Today, tan spot has become a major threat to sustainable wheat production with several epidemics in the post green revolution.

Eight races of *P. tritici-repentis* have been reported to date (Lamari et al., 2003) of which races 1 through 5 exist in the USA (Ali and Francl, 2003; Engle et al., 2006) with race 1 being the most prevalent in both the USA and worldwide (Ali and Francl, 2003). Three host-selective effectors (toxins: Ptr ToxA, Ptr ToxB and Ptr ToxC) produced by the fungus and considered to be associated with two distinctly identified symptoms (necrosis and chlorosis) were reported (Balance et al., 1989; Orolaza et al., 1995; Strelkove and Lamari, 2003; Tomas et al., 1990; Tuori et al., 1995). Ptr ToxA has been reported to serve as the pathogenicity or virulence factor (Friesen et al., 2006 and Tuori et al., 1995).

The change in tan spot status from a minor to major disease after the 1970's is believed to be the result of several factors including: i) adoption of zero tillage from conventional tillage practices in the 1970's, to avoid soil erosion, manage soil moisture, and save fuel costs in major wheat-growing countries including the USA. This change in tillage practice leaves a large amount of crop residue that may help *P. tritici-repentis* survive from one season to the next growing season (Conners, 1940); ii) an increase in

the pathogen virulence due to a horizontal transfer of *Ptr ToxA gene* from *Stagnospora nodorum* to *P. tritici-repentis* (Friesen et al., 2006; Oliver et al., 2008); iii) an accidental insertion of genes for tan spot susceptibility during the deployment of stem rust resistant cultivars after the 1930's and 1950's stem rust epidemics in the USA and other major wheat growing countries (DeWolf et al., 1998). Two studies have been conducted to find the link if any, between the genotype sensitivity to Ptr ToxA and the increase in tan spot in Australia (Oliver et al., 2008); and potential sources of susceptibility and sensitivity to Ptr ToxA in Canadian wheat and the increase in tan spot in the 1970's (Lamari et al., 2005), but mixed results were reported. To our knowledge, a global collection of wheat cultivars/genotypes developed prior to major stem rust epidemics in the USA during the 1930's and 1950's, including countries representing wheat origin/ center of diversity, have not been investigated for their reaction to tan spot and Ptr ToxA toxin. The objectives of this study were; i) to determine if stem rust resistance/ susceptibility is associated with increased susceptibility to tan spot; ii) to study if acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* is a major factor leading to increased susceptibility of bread and durum wheat to tan spot.

MATERIAL AND METHODS

Plant Materials

Two hundred and forty-seven wheat genotypes (bread wheat = 156; durum = 91) from to six continents: Africa, Asia, Australia, Europe, North America, and South America (Figure 2.1), which were developed and/or utilized commercially in thirty-nine countries prior to the 1930's and 1950's stem rust epidemics in the US Great Plains were obtained from USDA National Small Grains Collection, Aberdeen, Idaho. A few durum cultivars post 1970 from the USA were also characterized. Wheat genotypes Glenlea and Salamouni were included in the experiment as tan spot susceptible, Ptr ToxA sensitive and tan spot resistant, and Ptr ToxA insensitive checks, respectively. Two-week old seedlings of all 247 wheat genotypes including checks were raised in 5 x 23 cm containers (Stuewe & Sons, Inc. 31933 Rolland Drive, Tangent, Oregon 97389 USA) filled with Sunshine Mix 1 (770 Silver Street Agawam, MA, USA) and tested for their reaction to tan spot with *P. tritici-repentis* race 1, and sensitivity to Ptr ToxA. Nine seedlings (three seedlings/cone, with three rep) of each genotype were evaluated for reaction to tan spot and Ptr ToxA. The seedlings were watered, fertilized as needed, and were kept in a greenhouse at 21-22^{o}C with 16 hours' photoperiod until the experiment was terminated.

Figure 2.1. Origin of 247 wheat genotypes used in this study

Inoculum preparation, plants inoculation and disease rating

A single spore of *P. tritici-repentis* race 1 isolate "SD13-101-1" recovered from wheat in South Dakota was used for inoculum production throughout the experimentation. A fresh culture of the isolate was initiated by plating the isolate dry plugs stored at -20^{o}C on V8-PDA (V8 Juice =150 ml; $CaCO_3$ = 3 g; potato dextrose agar = 10 g; agar = 10 g; distilled water = 850 ml) to prepare spore suspension as described in Ali and Francl, 2001. Briefly, ten dry plugs (one plug/plate) were placed in the center on fresh V8-PDA plates, wrapped with aluminum foil paper, and incubated for 5-6 days at room temperature. After six days when the cultures had grown approximately 3 cm from the center, about 30 ml of distilled sterilized water was added into each plate, and the mycelial growth was knocked down with the help of a flamed sterile test tube bottom. The plates were incubated under fluorescent grow lights for 24 hours and then

in the dark at 16^{o}C for 24 hours to induce conidiophores and conidia development, respectively. Thereafter, about 30 ml of distilled sterile water was added to each plate, and conidia were collected with a loop wired needle and spore suspension was adjusted to 3000 spores/ml prior to inoculations.

Two-week old seedlings of each of genotypes were inoculated with the race 1 spore suspension with a CO_2 pressurized hand sprayer (Power Sprayer, Prevail, Chicago Aerosol, 1300 E. North Street, Coal City, IL60416) and placed in humidity chambers at 100% humidity for 24 hours for infection initiation. The seedlings were then moved from the humidity chambers to the greenhouse bench. Seven days' post-inoculations, the seedlings were rated for disease reactions using a 1 to 5 rating scale where lesion type 1-2 is resistant to moderately resistant and 3-5 is moderately susceptible to susceptible (Lamari and Bernier, 1989).

Screening for stem rust

Seedlings of 247 wheat genotypes were raised in jiffy pots containing potting soil. The screening was done at Cereal Disease Laboratory, USDA-ARS, St. Paul, MN. Seven to nine-day-old seedlings were inoculated with urediniospores when the first leaf was fully expanded. Seedlings were evaluated against a mixture of *P. graminis tritici* races (QFCSC, QTHJC, MCCFC, RCRSC, RKQQC, TPMKC, TTTTF, TTKST, TRTTF, and TKTTC) commonly prevalent in the US Great Plains and race TTKSK by following the method described by Jin et al. (2007). The seedlings were incubated in the humidity chamber at 18^{o}C for 14 hours in dark and 4 hours in light. The inoculated plants were placed on a

greenhouse bench at 18 ±2°C. The plants were rated for infection types (IT) described by Stakman et al. (1962).

Toxin bioassays

All bread and durum genotypes tested for tan spot above were evaluated for their reaction to Ptr ToxA (Appendix Table 2.1 and Figure 2.2). To avoid any discrepancy in screening results due to using different plants for the fungal and toxin reaction, nine fully expanded first leaves of each genotype, prior to inoculation with race 1 at two-leaf-stage, were infiltrated with purified Ptr ToxA @10 µg/ml using a needle-less syringe as described by Faris et al. (1996). The leaves were examined 72 hrs' post-toxin infiltration for necrosis development and rated as "+" (toxin sensitive) and "-" (insensitive). The purified Ptr ToxA was kindly provided by Dr. Steven Meinhardt, Department of Plant Pathology, North Dakota State University, Fargo, ND. Tan spot wheat differentials Glenlea (Ptr ToxA sensitive) and Salamouni (insensitive) were included as controls in the experiment. Three leaves of fifty randomly chosen genotypes were also infiltrated with toxin without race 1 spore inoculation and inoculated with race 1 spore suspension without the toxin infiltration to verify if the toxin infiltration into the first leaf would not impact the inoculation results. The supplementary tables and figures for wheat data can be accessed from http://tinyurl.com/z2j2mza.

RESULTS

Reaction of bread wheat genotypes to *P. tritici-repentis* race 1 and Ptr ToxA

156 bread wheat genotypes evaluated with tan spot (race 1) and reactions ranging from resistant to susceptible were observed. Of the 156 genotypes, 104 (66.6%) developed lesion type ranging from 3-5 and were rated moderately susceptible to susceptible to tan spot; whereas, 52 (33.3%) genotypes were rated from resistant to moderately resistant showing lesion type of 1-2 (Figure 2.2, appendix table 2.1). The same genotypes were also screened for reaction to Ptr ToxA; 51% (n=79) of the genotypes exhibited necrosis in the toxin infiltrated leaf area and were rated sensitive (Figure 2.2). The other 49% (n=77) were rated as toxin insensitive as they did not develop necrosis symptoms (Figure 2.2). The wheat genotypes that were rated as TS susceptible (n=104) were evaluated for ToxA reaction and 63% (n=65) were sensitive and 37% (n=39) were insensitive to Ptr ToxA. Whereas, 52 genotypes that exhibited resistance reaction to spore inoculations, 27% (n=14) were sensitive and 73% (n=38) were insensitive to Ptr ToxA (Figure 2.2 and 2.3).

		Bread wheat (156)		Durum wheat (91)	
		Ptr ToxA reaction			
		I (77)	Sen (79)	I (40)	Sen (51)
Tan spot reaction	S (104)	39 (37%)	65 (63%)	30 (42%)	41 (58%)
	R (52)	38 (73%)	14 (27%)	10 (50%)	10 (50%)
		a		b	

R- resistant; S- susceptible; I- Insensitive to Ptr ToxA; Sen- sensitive to Ptr ToxA

Figure 2.2. Reaction of a) bread wheat and b) durum wheat genotypes to tan spot (*P. tritici-repentis* race 1) and Ptr ToxA

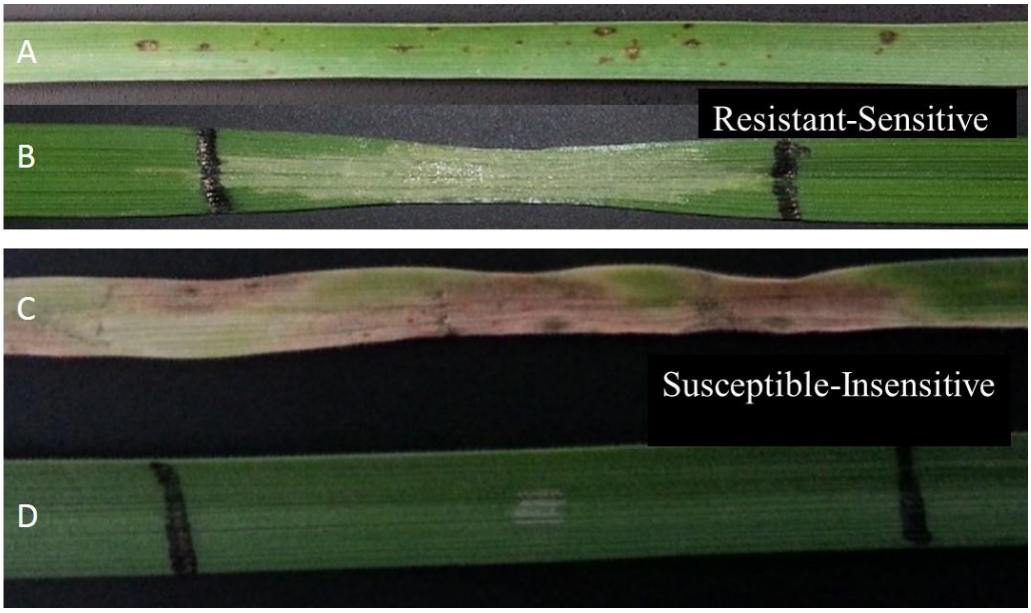

Figure 2.3. A) Bread wheat cultivar ('Molein Mis' released in 1923) showing resistance to fungus while inoculated with *P. tritici-repentis* race 1, B) exhibiting sensitivity to Ptr ToxA toxin, and C) Susceptible reaction with fungus to durum wheat line ('Capelli 38' released in 1946), D) but insensitive to Ptr ToxA toxin

When looking at the genotypes from different continents, 36% (n=14) of the wheat genotypes from Asia and Europe exhibited susceptibility to tan spot but were insensitive to Ptr ToxA (Table 2.1). However, an opposite trend was observed in susceptible genotypes belonging to Australia and North America where 56% (n=53) were sensitive to the toxin and susceptible to tan spot (Table 2.1). Seventeen percent (n = 16) of wheat genotypes exhibited resistance to race 1 and were also insensitive to the toxin.

Table 2.1. Reaction of 156 bread wheat genotypes from six continents to *P. tritici-repentis* race 1 and Ptr ToxA

Continent	Genotypes	R/I (%)	R/Sen (%)	S/I (%)	S/Sen (%)
Africa	16	7 (43.7)	3 (18.75)	4 (25.0)	2 (12.5)
Asia	16	2 (12.5)	0	9 (56.2)	5(31.2)
Australia	8	0	0	1(12.5)	7 (87.5)
Europe	23	11 (47.8)	4 (17.3)	5 (21.7)	3 (13.0)
N. America	86	16 (18.8)	6 (6.97)	18 (20.9)	46 (53.4)
S. America	7	2 (28.5)	1 (14.8)	2 (28.5)	2 (28.5)
Total	**156**	**38 (24.3)**	**14 (8.9)**	**39 (25.0)**	**65 (41.6)**

R = resistant, S= susceptible, Sen = toxin sensitive, I = toxin insensitive

Reaction of durum wheat genotypes to *P. tritici-repentis* race 1 and Ptr ToxA

In total, 91 durum genotypes were screened for their reaction to Ptr ToxA and tan spot using race 1 (produces Ptr ToxA). Nearly, 78% (n= 71) of the evaluated genotypes exhibited susceptibility to tan spot; whereas, the other 20 were resistant to

race 1 (Figure 2.2). All 91 genotypes were also evaluated for their reaction to Ptr ToxA, of which 56% (n= 51) turned out to be sensitive and the other 44% (n = 40) exhibited insensitivity to the toxin (Figure 2). Of 71 genotypes, susceptible to tan spot, 58% (n =41) and 42% (n=30) were sensitive and insensitive to Ptr ToxA, respectively (Figure 2.2 and 2.3). Half (n=10) of the genotypes that exhibited resistance to TS race 1 were also insensitive to the toxin, whereas other half were resistant to TS (race 1) but were sensitive to Ptr ToxA (Figure 2.2). Durum genotypes from North America were largely susceptible to tan spot and were also sensitive to the toxin; however, an opposite trend was observed in the genotypes belonging to Europe where 58% of genotypes were susceptible to the fungus but were insensitive to Ptr ToxA (Table 2.2). Of the durum genotypes from Africa that were rated as susceptible, 58% were sensitive and 42% insensitive to the toxin reaction (Table 2.2).

Table 2.2. Reaction of 91 durum wheat genotypes from six continents to *P. tritici-repentis* race 1 and Ptr ToxA

Continent	Genotypes	R/I (%)	R/Sen (%)	S/I (%)	S/Sen (%)
Africa	48	5 (10.4)	5 (10.4)	16 (33.3)	22 (45.8)
Asia	7	2 (28.8)	0	3 (42.8)	2 (28.6)
Australia	1	0	0	0	1 (100.0)
Europe	12	2 (16.7)	0	7 (58.3)	3 (25.0)
N. America	21	1 (4.7)	5 (23.80)	3 (14.2)	12 (57.1)
S. America	2	0	0	1 (50.0)	1 (50.0)
Total	**91**	**10 (11.7)**	**10 (11.4)**	**30 (34.1)**	**41 (43.61)**

R = resistant, S= susceptible, Sen = toxin sensitive, I = insensitive

Reaction of 247 wheat genotypes to stem rust

All 156-bread wheat and 91 durum genotypes were evaluated against stem rust using the bulk of local races. Nearly 99% (154) were susceptible with infection type (IT) ranging from (3-4) on the Stakman rating scale (Stakman et al., 1962). Only two genotypes developed IT of fleck -1 and were rated as resistant (Figure 2.4). Of these 154 stem rust susceptible genotypes, 64% (99) were rated as susceptible and 36% (55) resistant to tan spot (Figure 2.4). Additionally, comparing the reaction of stem rust susceptible genotypes (154) to Ptr ToxA demonstrated 53% (81) genotypes were sensitive and 47% (73) were insensitive to Ptr ToxA (Figure 2.4).

In durum wheat, 79% (72) of 91 genotypes were rated as stem rust susceptible and the other 21% (19) were resistant (Figure 2.4). Of those 72 stem rust susceptible genotypes, 75% (54) and 25% (18) were susceptible and resistant to tan spot, respectively. Also, 79% (53) of the stem rust susceptible genotypes were sensitive and the other 21% (19) were insensitive to the toxin Ptr ToxA (Figure 2.4). Of the 19 stem rust resistant durum genotypes, 79% (15) were susceptible, whereas, 21% (4) were resistant to tan spot. Of the stem rust resistant durums, 95% (18) durums showed sensitivity and 5% (1) exhibited insensitivity to the toxin when infiltrated with Ptr ToxA (Figure 2.4). In this study, we observed a large number of varieties/ genotypes that exhibited susceptibility to stem rust, also exhibit both resistant and susceptible reaction to tan spot and also display both sensitive and insensitive reaction to toxin Ptr ToxA; thus, a weak ($r=0.145$ for bread wheat and $r= -0.082$ for durum wheat) to no correlation was observed between the stem rust susceptibility and tan spot resistance.

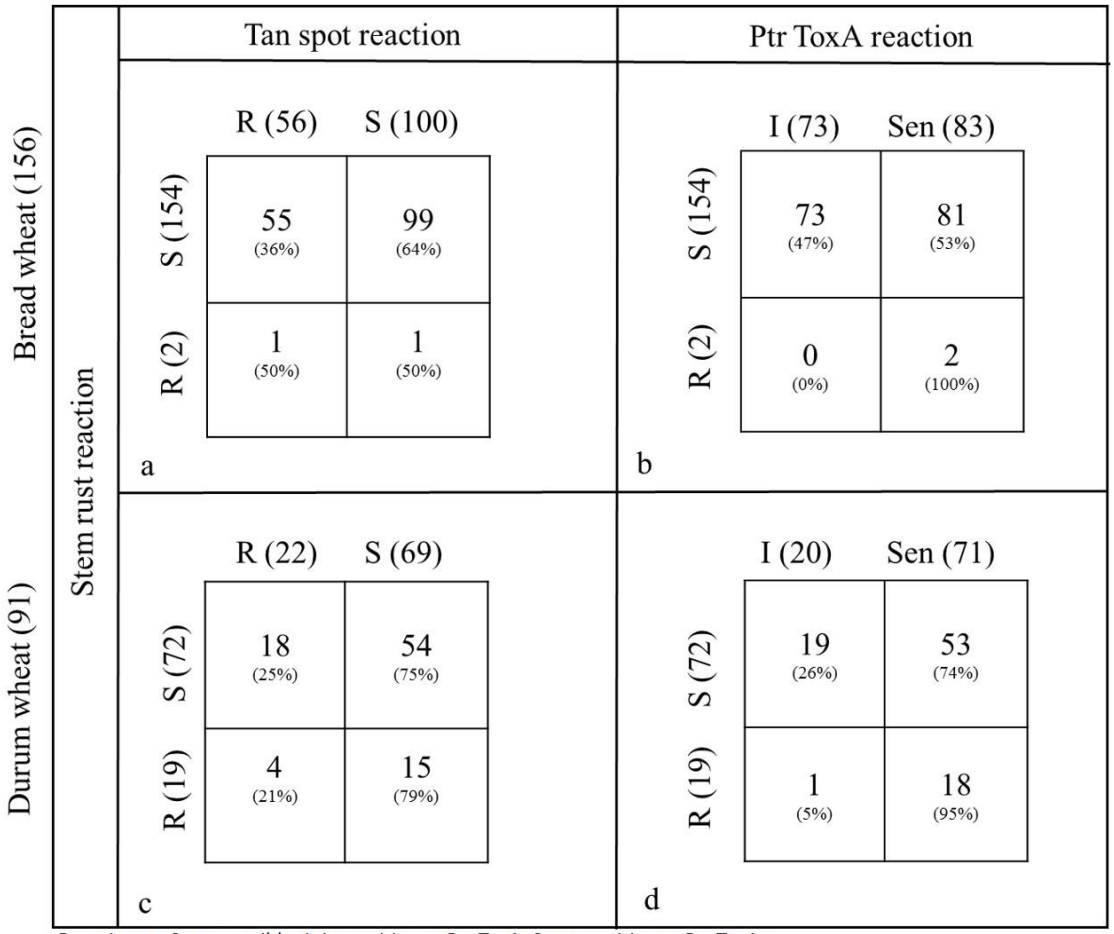

Figure 2.4. Comparative analysis of bread wheat genotypes for their reaction to, a) Tan spot and stem rust; b) Ptr ToxA and stem rust. Comparative analysis of durum wheat genotypes for their reaction to, c) Tan spot and stem rust; d) Ptr ToxA and stem rust.

DISCUSSION

A change in tan spot status from a minor disease prior 1960's to a major disease in the 1970's presents a serious threat to wheat production in the USA and the world. Plant pathologists and breeders are continuously developing superior cultivars with better resistance, and developing management strategies to minimize losses due to tan spot. An increase in tan spot occurrence could be a result of change in the pathogen virulence, and/or a gene insertion of susceptibility in the wheat varieties during deployment of stem rust resistance after rust epidemic in the 1930's and 1950's in the US and elsewhere (DeWolf et al., 1998). There is limited information on the reaction of bread wheat and durum wheat genotypes/cultivars developed prior to rust epidemics in the USA and elsewhere. Evaluating older genotypes for reaction to stem rust, tan spot, Ptr ToxA (toxin sensitivity), and the role of host selective toxins in the disease development can address two major questions; i) is stem rust resistance/ susceptibility associated with increased susceptibility to tan spot; ii) or, was acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* a major factor in increased susceptibility of wheat to tan spot? In this study, we evaluated pre-epidemic cultivars of bread wheat (n=156) and durum wheat (n=91), against stem rust, tan spot (*P. tritici-repentis* race 1), the Ptr ToxA producer and most prevalent race worldwide (Ali and Francl, 2003; Ali et al., 2010; Sarova et al., 2005). Our results did not show any correlation between stem rust susceptibility and tan spot resistance as well as the role of Ptr ToxA in the disease development. We observed multiple interactions where wheat cultivars were; i) stem rust susceptible and tan spot susceptible (Figure 2.4); ii) stem rust resistant and tan spot

resistant; iii) susceptible to race 1 and sensitive to the toxin; iv) resistant to race 1 and insensitive to the toxin; v) susceptible to the race 1 and insensitive to the toxin; and vi) resistant to the race 1 and sensitive to the toxin (Figure 2.4). A weak (r=0.145 for bread wheat and r= -0.082 for durum wheat) to no correlation between stem rust susceptibility and tan spot resistance and susceptibility was observed. Of the 154 wheat genotypes that were susceptible to stem rust, 55 (36 %) of them were resistant to tan spot and 99 (64 %) were susceptible, and similar results were observed in durum genotypes (Figure 2.4). Thus, the data suggests that resistance/ susceptibility to stem rust is not likely associated with an increased susceptibility to tan spot.

However, in a previous study (Lamari et al., 2005), 86 wheat genotypes/cultivars developed and deployed from pre-and post-stem rust epidemics in North America were screened against *P. tritici-repentis* races 2 and 5 and host-selective toxins Ptr ToxA and Ptr ToxB in Canada to investigate the sources of tan spot susceptibility and the toxins sensitivity (Lamari et al., 2005). They reported that sensitivity and susceptibility to tan spot may come from three bread wheat cultivars Red Fife, Hard Red Calcutta, Marquis, and one tetraploid wheat Yaroslav Emmer, utilized as a source of stem resistance in the development of first bread wheat cultivar “Hope” to combat stem rust in the US and beyond. All four cultivars were tan spot susceptible and Ptr ToxA sensitive. The majority of the cultivars were sensitive to race 5 and Ptr ToxB and a good correlation was observed between the genotype tan spot susceptibility and the toxin sensitivity, but why tan spot was not a problem before the 1970’s while most of the commercial cultivars were susceptible to tan spot was not explained or investigated. In contrast, our

results using a large collection of 247 genotypes did not follow this pattern as more than 50% of wheat genotypes exhibited susceptibility to race 1 (Ptr ToxA producers) but insensitivity to Ptr ToxA. In addition, some genotypes exhibited resistance to the fungal inoculation but were sensitive to the toxin.

In addition, we tested 247 wheat genotypes against stem rust, tan spot race 1, and Ptr ToxA, collected from various countries and continents with none of the genotype collection following any specific pattern; thus, indicating that stem rust resistance and tan spot susceptibility (race 1) and sensitivity to Ptr ToxA may not necessarily be always true in the wheat- tan spot host-pathogen system. Additionally, we identified wheat genotypes that exhibited resistance to spore inoculation but were sensitivity to Ptr ToxA; these genotypes may have an alternative mechanism for resistance to tan spot. Our results suggest that tan spot susceptibility-toxin(s) sensitivity and/or resistance-toxin insensitivity may not be ubiquitous (Lamari et al., 2005), and this discrepancy may be due to wheat genotypes collected and tested in the two studies may have been from different countries. The primary objective of Lamari et al. (2005) was to trace the sources of tan spot susceptibility that was incorporated into the Canadian wheat cultivars, and while ours were to understand if tan spot susceptible/sensitivity was incorporated in wheat while breeding for stem rust resistance and if acquiring *Ptr ToxA* gene from *S. nodorum* has any major role in the tan spot increase.

Further, if acquisition of Ptr ToxA from *S. nodorum* to *P. tritici-repentis* was a major factor leading to increased susceptibility of wheat to tan spot, most of the

genotypes should be expected to be sensitive to Ptr ToxA, however, we observed all four combinations (TS susceptible-Ptr ToxA insensitive, TS susceptible-Ptr ToxA sensitive, TS resistant-Ptr ToxA insensitive, TS resistant-Ptr ToxA sensitive) suggesting that Ptr ToxA was not the major factor responsible in increased incidence of tan spot (Figure 2.2). Our results further suggest that the role of toxin in the disease development is a genotype dependent host-pathogen interaction. Similar kinds of interactions have been observed among post 1970's modern bread wheat and durum wheat genotypes when they were challenged with race 1 or race 2 (both races produce Ptr ToxA) spore inoculation and Ptr ToxA (Ali et al., 2007; Ali et al., 2010; Friesen et al., 2003; Noriel et al., 2011).

The toxin Ptr ToxA might play a role in aggressiveness, not in pathogenicity was also suggested by Friesen et al. (2003) when wheat mutants (tan susceptible but ToxA insensitive) developed from Ptr ToxA sensitive bread wheat genotype ND495 showed slower development of disease initially in the mutants as compared to the wild type inoculated with race 2; however similar disease level was observed at the final disease rating time point. Another study (Andrie et aI., 2007) reported two isolates SO3 and PT82 which did not harbour *ToxA* and *ToxB* genes, respectively, but produced necrosis and chlorosis on wheat differential lines Glenlea (Ptr ToxA sensitive) and 6B662 (Ptr ToxB sensitive). These studies suggest there might be some other pathogenicity factors involved in addition to current host selective toxin (HST) which make the fungal population virulant or aggressive towards the 1970's. Our results, using genotypes older than 1970 shows that the all four combinations (TS susceptible-Ptr ToxA insensitive, TS

susceptible-Ptr ToxA sensitive, TS resistant-Ptr ToxA insensitive, and TS resistant-Ptr ToxA insensitive) existed pre-1970, validating our earlier observation (Ali et al., 2007; Ali et al., 2010) and other studies (Friesen et al., 2003, Noriel et al., 2011; Oliver et al., 2008), and suggesting that Ptr ToxA is likely not a major factor in tan spot increase in USA and elsewhere.

In our study, 64 % (n=14) of the bread wheat genotypes from Asia and Europe exhibited susceptibility to tan spot but were insensitive to Ptr ToxA. However, an opposite trend was observed in susceptible genotypes belonging to Australia and North America where 78% of susceptible genotypes were also sensitive to the toxin (Table 2.1). Similarly, in durum wheat, 81% (n=13) of the durum genotypes from North America and Australia that showed a susceptible reaction to the fungus were also sensitive to the toxin (Table 2.2). In Africa, tan spot susceptible and toxin insensitive cultivars were more prevalent. This suggest that primarily two types of germplasm, TS susceptible-toxin sensitive and TS susceptible-toxin insensitive, were utilized in the development of pre- and post stem rust epidemics durum and bread wheat cultivars globally; however, the ratio of the two germplasms utilized varied from continent to continent. Similar observations were reported by Noriel et al. (2011) while studying post 1970's genotypes. Additionally, the pathogen population with or without Ptr ToxA may have varied in different continents and thus decreased the toxin significance in tan spot development. Although tan spot has been reported from 6 continents, losses due to tan spot are greater in North America and Australia where the majority of the bread wheat genotypes were both susceptible to tan spot and sensitive to Ptr ToxA.

In another study (Ali et al., 2010), several isolates were identified which lacked *Ptr ToxA* gene but still induce necrosis, again suggesting Ptr ToxA was not the only factor responsible for tan spot incidence. Further, if insertion of Ptr ToxA from *S. nodorum* into *P. tritici-repentis* happened in the 1940's (Friesen et al., 2006), which is very recent event, it probably occurred on a very low scale. It may be very likely that the fungus can be transported over long distances through an infected seed, but it seems difficult if not impossible for the fungal population harboring Ptr ToxA to establish globally in such a short period. Several reports indicate the global prevalence of race 1, which characteristically shows both necrosis and chloroloris symptoms are due to Ptr ToxA and Ptr ToxC (Ali and Francl, 2003; Sarova et al., 2005; Singh et al., 2010.) However, the pathogen population prevalent prior to the 1940's needs to be further characterized to understand of virulence mechanism in tan spot and if it carried Ptr ToxA. Our study showed TS susceptible and sensitive varieties were widely grown prior to the green revolution. Existence of wheat genotypes and their diverse reaction to the fungal isolates for tan spot symptoms (necrosis) with or without ToxA in our current study indicate a low association in tan spot outbreaks in the 1970's and later.

A tan spot epidemic was observed in 1937 and 1939 in Manitoba, and in 1941 in Saskatchewan (Conners, 1940 and 1941). The upsurge in disease in those years were thought to be due to the use of a one-way disk for soil preparation which may have left a lot of residue on the soil which favored the pathogen overwintering. Similar observations on an increase in tan spot and other stubble borne wheat diseases were reported when farmers adopted zero tillage, moving away from conventional tillage

practices to avoid soil erosion, improve moisture retention, and save on fuel expenses (Hosford, 1982; DeWolf et al., 1998; Odvody et al., 1982). Suggesting retention of infested crop residue due to a change in tillage practices may have a significant role in upsurge of tan spot. Our study shows neither incorporation of stem rust resistance during breeding nor horizontal transfer Ptr ToxA led to increased susceptibility of wheat cultivars in northern Great Plains.

ACKNOWLEDGEMENTS

We thank Dr. Steven Meinhardt for providing purified Ptr ToxA and Navjot Kaur for her help in greenhouse and lab work. SDSU Agricultural Experiment Station (AES) and South Dakota Wheat Commission provided financial support for this research.

LITERATURE CITED

Ali, S. and Francl, L. J. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. *Plant Dis.* 85: 580-584.

Ali, S. and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses. *Plant Dis.* 87:418-422.

Ali, S., Adhikari, T. B., Friesen, T. L., Meinhardt, S. and Rasmussen, J. B. 2007. A wheat genotype sensitive to Ptr ToxA and resistant to *Pyrenophora tritici-repentis* (Tan spot) races 1 and 2. *Phytopathology* 97: S3.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Dis.* 94:229–235.

Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007. A combination of phenotypic and genotypic characterization *Pyrenophora tritici-repentis* race identification. *Phytopathology* 97:694-701.

Balance, G. M., Lamari, L., and Bernier, C. C. 1989. Purification and characterization of a host-selective toxin from *Pyrenophora tritici-repentis*. *Physiol. Mol. Plant Pathol.* Online, publication/1996/1209/balance.

Barrus, M. F. 1942. Yellow spot disease of wheat in New York State. Plant Dis. Rep. 26:246.

Conners, I. L. 1932. In: 12th Annu. Rep. Can. Plant Dis. Surv. Canada Department of Agriculture, Experimental Farms Branch. P 8.

Conners, I. L. 1940. In: 20th Annu. Rep. Can. Plant Dis. Surv. Canada Department of Agriculture. Division of Botany and Plant Pathology. P 3.

Conners, I. L. 1941. In: 21st Annu. Rep. Can. *Plant Dis.* Surv. Canada Department of Agriculture. Division of Botany and Plant Pathology. P 6.

De Wolf, E. D., Effertz, R. J., Ali, S., Francl, L. J. 1998. Vistas of tan spot research. *Can. J Plant Pathol.* 20: 349–370.

Diedicke, H. 1902. Uber den *Zusammenhang zwischen* Pleospora— und *Helminthosporium*—Arten. Centralblatt für Bakteriologie und Parasitenkunde Jena Abt 9:317–329.

Engle, J. S., Madden, L. V., and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology* 96:1355-1362.

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora* tritici-repentis. *Phytopathology* 86:459- 463.

Friesen, T. L., Stucken Brock, E. H., Liu, Z. H., Meinhardt, S., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A., and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetic* 38:953-956.

Friesen, T. L., Ali, S., Kianian, S., Francl, L. J. and Rasmussen, J. B. 2003. Role of host sensitivity to Ptr ToxA in the development of tan spot. *Phytopathology* 93:397-401.

Hosford, R. M. 1982. Tan spot - developing knowledge 1902-1981, virulent races and wheat differentials, methodology, rating systems, other leaf diseases, literature. In R. M.

Hosford (Ed.), Tan Spot of Wheat and Related Disease Workshop (pp. 1-20). Fargo, ND: North Dakota Agric. Exp. Station.

Jin, Y., Singh, R. P., Ward, R. W., Wanyera, R., Kinyua, M., Njau, P., Pretorius, Z. A., and Yahyaoui, A. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp *tritici*. *Plant Dis.* 91:1096–1099.

Krupinsky, J. M. 1992. Grass hosts of *Pyrenophora tritici repentis. Plant Dis.* 76:92–95.

Lamari, L. and Bernier, C. C. 1989. Evaluation of wheat for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. *Can. J. Plant Pathol.* 11:49-56.

Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. *Phytopathology* 93:391-396.

Lamari, L., McCallum, B. D., and DePauw, R. M. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. *Phytopathology* 95:144-152.

Mitra, M. 1934. A leaf spot disease of wheat caused by *Helmiminthosporium tritici-repentis* Died. *Indian J. of Agri. Sci.* 4:692-700. 1934.

Moreno, M. V., Stenglein, S. A., and Perelló, A. E. 2012. *Pyrenophora tritici-repentis*, Causal Agent of Tan Spot: A Review of Intraspecific Genetic Diversity, The Molecular Basis of Plant Genetic Diversity, Prof. Mahmut Caliskan (Ed.), ISBN:978-953-51-0157-4.

Nisikado, Y. 1928. Preliminary notes on yellow leaf spot diseases of wheat caused by *Helminthosporium tritici-vulgaris.* Nisikado. *Inst. Agric. Biol.* 4:103-109.

Noriel, A. J., Xiaochum, S. Willimam, B., and Guihua, B. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. *Crop Science* 51:1059-1067.

Odvody, G. N., Bossalis, M. G., and Watkins, J. E. 1982. Development of pseudothecia during progressive saprophytic colonization of wheat straw by *Pyrenophora trichstoma*. Pages 33-35 in R. M. Hosford, Jr. ed. Tan spot of wheat and related diseases workshop. North Dakota agric. Exp. Station, Fargo, ND.

Oliver, R. P., Lord, M., Rybak, K., Faris, J. D., and Solomon, P. S. 2008. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin sensitive cultivars. *Phytopathology* 98:488-491.

Orolaza, N., Lamari, L. and Balance, G. M. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal organism of tan spot of wheat. *Phytopathology* 85:1282-1287.

Rees, R. G., and Platz, G. J. 1983. Effect of yellow spot on wheat: Comparison of epidemics at different stages of crop development. *Aust. J. Agric. Res.* 34:39-46.

Sarova, J., Hanzalova, A., and Bartos, B. 2005. Races of *Pyrenophora tritici-repentis* in Czech Republic. *Acta Agrobotanica* 58:73-78.

Schilder, A. M. C., and Bergstrom, G. C. 1995. Seed transmission of *Pyrenophora tritici-repentis*, causal fungus of tan spot of wheat. *Eur. J. Plant Pathol.* 101:81-91.

Singh, P. K., Singh, R. P., Duveiller, E., Mergoum, M., Adhikari, T. B., and Elias, E. M 2010. Genetics of wheat-*Pyrenophora tritici-repentis* interactions. *Euphytica* 171:1–13.

Shabeer, A., and Bockus, W. W. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Dis.* 72:599-602.

Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press Co., New York. 538 pp.

Stakman, E. C., Stewart, D. M., and Loegering, W. Q 1962. Identification of physiological races of *Puccinia graminis var. tritici*. United States Department of Agriculture, Agricultural Research Service E-617.

Strelkov, S. E., and Lamari, L. H. 2003. Host-parasite interactions in tan spot *Pyrenophora tritici-repentis* of wheat. *Can. J Plant Pathol.* 25:339–49.

Tomas, A., Feng, G. H., Reech, G. R., Bockus, W. W., and Leach, J. E. 1990. Purification of a cultivar-specific toxin of *Pyrenophora triticirepentis*, causal agent of tan spot of wheat. *Mol. Plant-Microbe Interact.* 3:221-224.

Tuori, R. P., Wolpert, T. J., and Ciuffetti, L. M. 1995. Purification and immunological characterization of toxic components from cultures of *P. tritici-repentis*. *Mol. Plant-Microbe Interact.* 8:41-48.

CHAPTER 3

TITLE: CHARACTERIZATION OF *PYRENOPHORA TRITICI-REPENTIS* (TAN SPOT OF WHEAT) RACES IN BALTIC STATES AND ROMANIA

Abdullah, S., Sehgal, S. K., Ali, S., Liatukas, Z., Ittu, M., and Kaur, N. 2017. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Baltic States and Romania. *Plant Pathol. J.* 33(2):133-139.

ABSTRACT

Tan spot, caused by the fungus *Pyrenophora tritici-repentis,* is an economically important foliar disease in Latvia, Lithuania, and Romania. However, race structure of the pathogen from Baltic States and Romania is not known. In this study, we performed genotypic and phenotypic race characterization of a large collection of *P. tritici-repentis* isolates from these countries to determine race structure and utilize this information for better disease management and breeding wheat for tan spot resistance. We characterized 231 single spore isolates from Latvia (n = 15), Lithuania (n = 107), and Romania (n = 109) for *Ptr ToxA* and *Ptr ToxB* genes using two genes specific primers. Subsets (139) of 231 isolates were further characterized for their race structure by inoculating them individually on tan spot wheat differentials set. Majority (83%) of the 231 isolates amplified *Ptr ToxA* gene suggesting prevalence of race 1 and 2. Further, phenotypic characterization of 139 isolates also showed wide prevalence of races 1 (68%), 2 (8%), 3 (11%), and 4 (5%) were also identified from Baltic States as well as Romania. Eighteen of the isolates (13%) did not seem to be of any of the eight known races as they lacked *Ptr ToxA* gene but they behaved like either race 1 or race 2, suggesting possibility of novel toxins in these isolates as their virulence tools.

Key words: Tan spot, *Triticum aestivum*, Baltic states, race, virulence genes

INTRODUCTION

Pyrenophora tritici-repentis (tan spot of wheat) races in Baltic States and Romania

Wheat is ranked first among small grains production in the Baltic States and Romania. In 2015, wheat was planted on 0.85, 1.97, and 5.11 million acres with 46, 160, and 307 million bushels' production in Latvia, Lithuania, and Romania, respectively (USDA Foreign Agric. Service 2016). Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died) Shoem, is an important leaf disease of wheat in the Baltic States (Latvia and Lithuania), Romania, and in many other wheat-growing countries (Beruta and Ilze, 2011; DeWolf et al., 1998; Hosford, 1982; Ronis et al., 2009). The disease was observed in the early 1990's at insignificant level in Baltic States and Romania and since then it is on the rise in the region (Beruta and Ilze, 2011). Increase in tan spot in these countries is thought to be the result of a large acreage of wheat grown under high yielding cultivars but potentially susceptible to tan spot, shift in cultural practices from conventional to minimum tillage that provide ample opportunity for residue borne pathogens like *P. tritici-repentis* inoculum buildup, and monoculture (Beruta and Ilze, 2011). Tan spot can cause yield losses from 3-50 percent (Shabeer and Bockus, 1988). In Lithuania, 73% reduction in one thousand kernels weight (TKW) has been reported depending on the cultivar susceptibility level and inoculum level, and suitable weather conditions for disease development (Beruta and Ilze, 2011; Ronis et al., 2009).

Tan spot can primarily be managed through fungicide application; however, deployment of resistant cultivars seems to be a more economical, environmentally safe

and durable approach. Excessive use of fungicide as a disease management strategy could create selection pressure leading to evolution of fungicide resistance in a pathogen population. Fungicide resistance in *P. tritci-repentis* population has been documented from various countries including Germany and Denmark (Sierotzki et al., 2007).

Development and deployment of durable disease resistant cultivars requires complete information about the pathogen virulence. Variation in *P. tritici-repentis* virulence has been observed based on using both quantitative, i.e., % necrotic leaf area, lesion size, and lesion number or combination of all the three components (Krupinsky, 1992; Luz and Hosford, 1980; Misra and Singh, 1972; Schilder and Bergstrom, 1990), and qualitative (i.e., lesion type (Lamari et al., 2003)) rating scales. Lamari and Bernier (1989) for the first time observed two distinct leaf spot symptoms, tan necrosis and chlorosis, produced by *P. tritici-repentis* on susceptible wheat genotypes. Based on these two distinct symptoms, they developed a pathotype classification system for detecting physiological variation in the fungal population. They grouped the isolates into four pathotypes based on their ability to produce necrosis (nec+) and chlorosis (chl+) and designated them as pathotype 1 (nec+chl+), 2 (nec+chl-), 3 (nec-chl+) and 4 (nec-chl-) based on their reaction on four bread wheat genotypes, Glenlea, 6B365, 6B662, and Salamouni. Later on, some *P. tritici-repentis* isolates were found that also produced chlorosis on 6B662 instead 6B365 of the four genotypes and could not be grouped under any of the four identified pathotypes (Lamari et al., 1995). To accommodate these *P. tritici-repentis* isolates with new virulence, they renamed the pathotype system as

race classification system (Lamari et al., 2003). They designated the pathotypes 1- 4 as races 1- 4 and the newly identified isolates as race 5. The race classification is well-accepted system by the research community working on this pathogen and it has enhanced the researchers' ability to understand wheat-tan spot host pathogen system.

Till to date, eight races have been identified in the pathogen populations. Three host-selective toxins Ptr ToxA, Ptr ToxB, and Ptr ToxC have been isolated from the fungal isolates that are associated with necrosis and chlorosis symptoms development and serve as either pathogenicity or aggressiveness factors (Ciuffetti and Tuori, 1999; Frisen et al., 2003). Of these three host-selective toxins, Ptr ToxA and Ptr ToxB are protenious in nature and have been sequenced and the toxins gene specific primers are available for studying the genotypes of known *P. tritci-repentis* isolates (Andrie et al., 2007). In contrast to these two toxins, Ptr ToxC is a non-ionic, polar, and low molecular weight molecule (Effertz et al., 2002). Ptr ToxC has not been purified, the gene encode it has been not cloned; it is not possible to characterize the fungal isolates for presence of *Ptr ToxC* gene without testing them on a Ptr ToxC sensitive differential line 6B365.

Several independent studies conducted on the physiological variation in *P. tritici-repentis* populations have observed diversity in race structure from various countries (Aboudkhaddour et al., 2013; Ali and Francl, 2001; Ali and Francl, 2002; Ali et al., 2010; Banslimane et al., 2011; Engle et al., 2006; Gamba et al., 2012; Lamari and Bernier, 1989; Lamari et al., 2003; Lepoint, et al., 2010; Moreno et al., 2008; Mikhalova et al., 2014; Sarova et al., 2005). Globally race 1 was the most prevalent race (Ali and Francl, 2003; Lamari et al., 2003), however, six races 1, 4, 5, 6, 7, and 8 were reported from

Algeria. In USA and Canada races 1-5 have been observed (Ali and Francl, 2002; Ali and Francl, 2003; Engle et al., 2005; Lamari and Bernier, 1989; Lamari et al., 2003), whereas, races 1 and 2 were observed in South America (Gamba et al., 2012; Mariono et al., 2005).

Since tan spot has been observed in the early 1990's, wheat breeding programs in Baltic states and Romania have developed and released few moderately resistant tan spot cultivars but the disease still impacts crop productivity significantly under high disease pressure (Liatukas et al., 2012). Occurrence of multiple races in the pathogen populations and resistance to individual race controlled by independent genes in the host warrants the investigation of virulence variation in the pathogen populations in the region for development of durable tan spot resistant cultivars. To our knowledge, information on *P. tritici-repentis* races prevalent in the Baltic States and Romania on wheat is not available. In this study, we have characterized the fungal isolates from Baltic States and Romania for their race structure using both phenotypic and genotypic approaches.

MATERIAL AND METHODS

Fungal isolates

Two hundred and thirty-one *P. tritici-repentis* isolates from Lithuania (n=107), Latvia (n = 15) and Romania (n = 109) were used for their race structure in this study (Appendix table 3.1). The isolates were provided by Dr. Z. Liatukas and were received under the APHIS permit number P526P-13-02732. The fungal isolates were recovered from tan spot infected leaves samples collected from various wheat growing regions of Lithuania, Latvia, and Romania in 2013 and 2014. Single spore cultures of all 231 isolates were obtained prior to studying their race structure. To obtain single-spore cultures, the isolates were individually grown on V8-PDA medium and conidia were produced as described by Lamari and Bernier (1989). The conidia of each isolate were spread by cutting and rubbing an about 2 cm block of V8-PDA with conidia on fresh V8-PDA plates and single spores were removed with a flamed scalper under stereoscope and grown on a V8-PDA plate. The isolates were stored at -20^{o}C by following the protocol of Jordhal and Francl (1992) until characterized for their race structure.

Molecular characterization of the isolates for Ptr ToxA and Ptr ToxB

DNA extraction and PCR assay

DNA of all 231 *P. tritici-repentis* isolates was obtained by initiating fresh cultures individually on V8-PDA medium by plating frozen dry plugs stored at -20^{o}C and grew them for 5 days. The mycelia were scraped from the agar surface using a flamed scalpel and placed in a 2-ml microfuge tube. The mycelia was then dried overnight in a water bath at 37°C and then ground into a fine powder using a first prep machine (Retsch MM

301). DNA was extracted from mycelia of each isolate by following the procedure of Moreno et al. (2008). The DNA concentration normalized to 25ng/µl using a Nano drop machine (NanoDrop 1000 UV/Vis Spectrophotometer, Counterpane Inc. Tacoma, WA, USA) and run in a 0.8% agarose gel to verify the DNA quality. Genotype of the *P. tritici-repentis* isolates for *Ptr ToxA and Ptr ToxB* genes were determined by using the *Ptr ToxA* and *Ptr ToxB* genes specific primers developed by Andrie et al. (2007). The conformity of the isolates of *P. tritici-repentis* was determined by using two *P. tritici-repentis* mating type genes specific primers suggested by Lepoint et al. (2010). PCRs for specific markers were performed in 20 µl volume; 2 µl genomic DNA (25ng/ µl, 0.8 µl of each primer (10mM), 0.5 µl dNTP (200 µM), 2 µl 10 x thermophol buffer, 0.2 µl 10 U/ml *Taq* Polymerase and 13.7 µl of molecular biology water. PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA) using amplification steps of 94°C for 1 minute, followed by 30 cycles of 94°C for 45 seconds, 55°C for 30 seconds and 72°C for 1 minute with final extension of 72°C for 7 minutes. The amplified products were electrophoresed on 1.5% agarose gels and scored with reference to 1 Kb ladder (New England Biolabs, USA). Two known *P. tritici-repentis* isolates, one each of race 1 and race 5 were used in the experiment as positive checks for *Ptr ToxA* and *Ptr ToxB* genes.

Phenotypic characterization of 139 *P. tritici-repentis* isolates

Seedling production of tan spot wheat differentials and standard fungal isolates

Four tan spot wheat differential genotypes Glenlea, 3B365, 6B662, and Salamouni seedlings were raised in 3 x 9 cm plastic containers (Stuewe & Sons, Inc. 31933 Rolland Drive, Tangent, OR 97389 USA) filled with Sunshine Mix 1 (770 Silver

Street Agawam, MA, USA). Three seedlings/container were maintained throughout the experimentation. The plants were watered daily and fertilized once every 10 days post-planting using a slow releasing fertilizer. The plants were kept on a greenhouse bench at 22^{o}C set at 16 hours' light and 8 hours' dark cycle. The fungal isolates SD13-101-1 (race 1) and SD13-103 (race 5) recovered from tan spot infected wheat leaves collected in South Dakota were included as positive checks for validation of inoculation and race identification.

Inoculum, inoculation and race identification

Inoculum preparation and inoculations were done as described in Ali and Francl (2001). Briefly, fresh cultures of randomly selected 139 of the 231 isolates genotyped for *Ptr ToxA* and *Ptr ToxB* genes were initiated by plating their frozen dry plugs on to fresh V8-PDA medium plates (agar = 10 grams; PDA = 10 grams; $CaCO_3$ = 3 grams; V8-Juice =150 ml and 850 distilled water) (Lamari and Bernier, 1989). The plates were incubated in dark for 5 days (when the fungal colony growth generally reaches to 4-5 cm in diameter). The plates were then flooded with distilled sterile water and the hyphal growth was knocked down with the help of a flamed bottom of a test tube. Excess water was removed from the plates prior to incubating them for 24 hours in light at room temperature (~22^{o} C) and then at 16^{o}C for 24 hours in dark for conidial production. The spores were collected with a flamed looped wire by adding about 30 ml of distilled sterile water in each plate and spore concentration was adjusted @ 2500 spores/ml as described by Jordahl and Francl (1992).

Two-week old seedlings of all 4 differentials genotypes with three replications were inoculated individually with all 139 isolates by spraying their spore suspension @2500 spores/ml using a CO_2 pressurized sprayer (www.Preval.com) and the inoculated seedlings were placed for 24 hours in an automated humidity chamber set at 100% humidity by misting 16 seconds/10 minutes for enhancing the chances of fungal infection. Thereafter, the seedlings were moved to greenhouse until rated for symptom development. The isolates were grouped under appropriate race based on their ability to produce necrosis and chlorosis symptoms on appropriate tan spot wheat differentials set developed by Lamari et al. (2003) (Table 3.1).

Table 3.1. Races of *Pyrenophra tritici-repentis* and their symptoms on tan spot wheat differentials set (Lamari et al., 2003)

		Wheat differential lines			
Races	**Ptr Toxin Genes**	**Glanlea (Sensitive to ToxA)**	**6B365 (Sensitive to ToxC)**	**6B662 (Sensitive to ToxB)**	**Salamouni (Insensitive to Toxin A, B and C**
Symptom →		**Necrosis** 	**Chlorosis** 	**Chlorosis** 	**Avirulent** 
1	*ToxA* and *ToxC*	Necrosis	Chlorosis	Avirulent	Avirulent
2	*ToxA*	Necrosis	Avirulent	Avirulent	Avirulent
3	*ToxC*	Avirulent	Chlorosis	Avirulent	Avirulent
4	Nil	Avirulent	Avirulent	Avirulent	Avirulent
5	*ToxB*	Avirulent	Avirulent	Chlorosis	Avirulent
6	*ToxB* and *ToxC*	Avirulent	Chlorosis	Chlorosis	Avirulent
7	*ToxA* and *ToxB*	Necrosis	Avirulent	Chlorosis	Avirulent
8	*ToxA, ToxB* and *ToxC*	Necrosis	Chlorosis	Chlorosis	Avirulent

RESULTS AND DISCUSSION

Pyrenophora tritici-repentis isolates characterization for Ptr ToxA and Ptr ToxB genes

P. tritici-repentis mating-type specific DNA bands were amplified in all 231 isolates analyzed conforming the identity of the isolates. As expected, 585bp and 295bp DNA bands associated with *Ptr ToxA* and *Ptr ToxB* genes, respectively were amplified from the check isolates validating the PCR assay (Figure 3.1, Table 3.2). Eighty-three percent (n =191) of the isolates harbored *Ptr T*oxA gene (Figure 3.1, 3.2), however, none of the 231 isolates amplified *Ptr ToxB* gene. Out of the 231 isolates analyzed, 17% (n = 40) did not harbor either of the two toxin genes. Genotypic characterization suggests prevalence (83%) of *Ptr ToxA* harboring populations (races 1, 2, and 7) in Baltic countries and Romania. The lack of *Ptr ToxB* gene in the isolates eliminates the possibility of races 5, 6, and 8 in the region as these races contain the *ToxB* gene (Lamari et al., 2003). The other 17% (n = 40) of the isolates lacked both *ToxA* and *ToxB* genes indicating the existence of either race 3 (nec-chl+) and/or 4 (nec-chl-). Another possibility is that these isolates potentially harbor new virulence genes as previously reported by Ali et al. (2010) and Andrie et al. (2008). They also identified some isolates that behaved like race 1 and race 5 but lacked *ToxA* and *ToxB* genes. Due to lack of good molecular markers for *Ptr ToxC* gene(s), discriminating the fungal isolates that carry *Ptr ToxC* gene(s) is presently possible by testing isolates on tan spot differential set for their race identification.

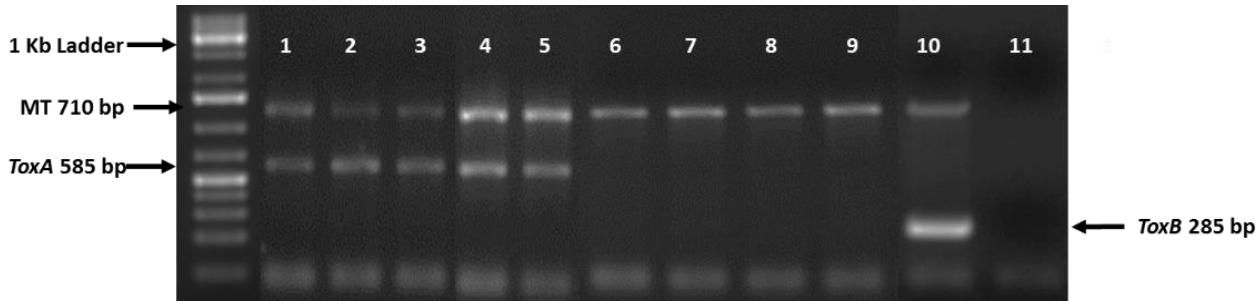

Figure 3.1. Gel picture of the *P tritici-repentis* isolates from Latvia, Lithuania, and Romania showing the presence/absence of *ToxA*, *ToxB* and *Mating type* (*MT*) gene Lane 1-3: *Ptr ToxA* gene in 14-LV-2, 13-LT-5-9, and 14 RO-5-17 isolates (race 1); lane 4-5: *Ptr ToxA* gene in 13-LT-1-11 (race 2) and Pti2 of race 1 (positive control); lane 6-7: Isolate from LT as race 3 and race 4; lane 8-9: Necrosis producing isolates 13-LT-2-10 and RO-5-23 without *ToxA gene*; lane 10-11: race 5 isolate DW7 (positive control for *Ptr ToxB*) and water as negative control, respectively

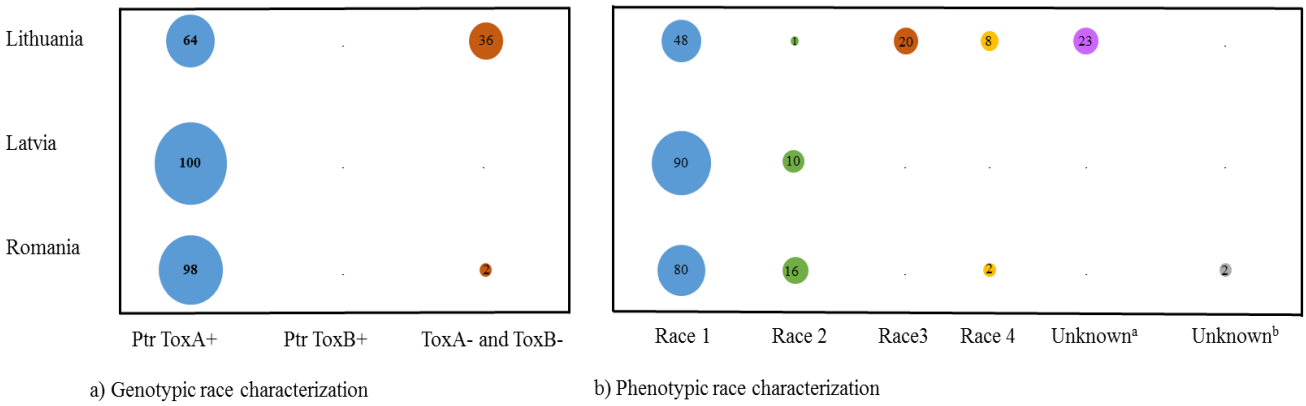

Figure 3.2. a) Genotypic characterization of 223 *P tritici-repentis* isolates recovered from wheat for *Ptr ToxA* and *Ptr ToxB* genes from Latvia, Lithuania, and Romania the percent of races classified as carrying *Ptr ToxA* / *Ptr ToxB* gene or none of them b) Race characterization of 139 *P tritici-repentis* isolates recovered from wheat collected in Latvia, Lithuania, and Romania. The percent of races classified as race 1, race 2, race3, race 4 or unknown races

Table 3.2. Genotypic characterization of 231 *P. tritici-repentis* isolates recovered from wheat for *Ptr ToxA* and *Ptr ToxB* genes from Latvia, Lithuania, and Romania

Country	Isolates tested	*Ptr ToxA*+	*Ptr ToxB*+	*Ptr ToxA*- and *ToxB*-
Latvia	15	15	0	0
Lithuania	107	69	0	38
Romania	109	107	0	2
Total	**231**	**191**	**0**	**40**

+ = Toxin gene present; - = Toxin gene absent

Race identification of *P. tritici-repentis* isolates from Latvia, Lithuania, and Romania

A subset of 231 isolates genotyped for *Ptr ToxA* and *Ptr ToxB* were evaluated on four differentials to obtain a phenotypic reaction of 139 isolates. A diverse fungal population was prevalent in three countries based on our phenotypic evaluation (Figure 3.2). Overall, 63% (n = 88) of the isolates were grouped under race 1 as they produced necrosis and chlorosis on Glenlea and 6B365, respectively and neither symptoms on 6B662 and Salamouni whereas, 8% (n = 11) of the phenotyped isolates were designated as race 2 because they produced necrosis on Glenlea and resistant reaction on the other three differential genotypes. About 11% (n = 15) of the isolates induced chlorosis on 6B365 and neither necrosis or chlorosis on Glenlea, 6B662, and Salamouni and were designated as race 3. Only 5% (n = 7) of the isolates were grouped under race 4, as they did not produce either of the symptoms on all four differential genotypes. Thirteen percent (n =18) of the isolates could not fit under any of the currently identified 8 races as 17 of them produced necrosis and chlorosis on Glenlea and 6B365 like race 1 but they lacked *Ptr ToxA* gene. One isolate (RO 5-23) exhibited reactions like race 2 as it produced necrosis on Glenlea and no symptoms on 6B365, 6B662, and Salamouni (Figure 3.3), however, lacked *Ptr ToxA* gene. Thus, fungal isolates collected from

Lithuania were grouped into races 1, 2, 3, 4, and additional isolates (23%) with different virulence pattern from the currently identified eight races. Race 1 (48%) and race 3 (20%) were the most prevalent races in Lithuania (Figure 3.2, Table 3.3) followed by race 4 (8%). Of the fungal isolates from Romania, 80% were grouped as race 1 (n = 43), 17% as race 2 (n = 10), and 2% as race 4 (n = 1). Only one isolate that lacked *ToxA* gene and behaved like race 2 could not fit under any of the currently identified 8 races. Our results indicate the presence of races 1, 2, and 3 with race 1 the most prevalent race in the region. Presence of races 1-3 on wheat in Balkan state can be expected as all three races with race 1 and 2 at higher frequency were observed in its neighboring country Russia (Mikhalova et al., 2014). The fungal spores can travel long distance through wind (Francl, 1997) and establish far apart from the source of origin under suitable weather conditions. Weather conditions are similar in wheat growing areas on both sides of the border sharing Russia and Baltic State countries. Prevalence of race 1 as a predominant race in the region is not very surprising, this has been observed as the most prevalent race in Algeria (Benslimane et al., 2011), Czech Republic (Ali et al., 2004; Sarova et al., 2005), Canada (Aboudkhaddour et al., 2013; Lamari and Bernier 1989; Singh and Hughes 2006), USA (Ali and Francl, 2003; Engle et al., 2005), and South America (Ali and Francl, 2002; Gamba et al., 2012; Moreno et al., 2008). Prevalence of race 1 in higher frequency suggests that wheat germplasm used in the three countries has narrow diversity as far as susceptibility to tan spot races is concerned. Evaluation of both bread wheat and durum wheat genotypes for tan spot using race 1 and race 5 in two independent studies also found higher susceptibility to race 1 as compared to race 5. (Ali et al., 2008; Singh et

al., 2006). Prevalence of race 4 in low frequency (5%) in the three countries is likely as this race does not contain any of the three-known host-selective toxins and is avirulent on wheat that makes it less competitive with other races for its establishment. Similar trend of prevalence of race 4 at low frequency was reported on wheat from other countries as well (Ali and Francl, 2003; Benslimane et al., 2011; Lamari and Bernier 1989; Mikhalova et al., 2014, and Sarova et al., 2005).

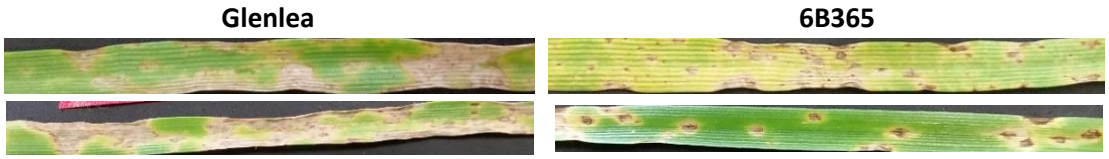

Figure 3.3. Two *P tritici-repentis ToxA*- isolates 13-LT-2-10 produced necrosis on Glenlea and chlorosis on 6B365 (left panel top and right panel top) and RO-5-23 produced necrosis on Glenlea and resistant reaction on 6B365 (left panel bottom and right panel bottom) behaved like race 1 and 2, respectively

Table 3.3. Phenotypic race characterization of 139 *P. tritici-repentis* isolates recovered from wheat collected in Latvia, Lithuania, and Romania

Country	Isolates tested	Race 1	Race 2	Race 3	Race 4	Unknown[a]	Unknown[b]
Latvia	10	9	1	0	0	0	0
Lithuania	75	36	1	15	6	17	
Romania	54	43	9	0	1	0	1
Total	**139**	**88**	**11**	**15**	**7**	**17**	**1**

[a] = *P. tritici-repentis* isolate(s) lacks in Ptr ToxA gene but behave like race 1; b = *P. tritici-repentis* isolate lack in ToxA gene but behaves like race 2.

Interestingly, 13% (n = 17) and 2% (n = 1) of the isolates from Lithuania and Romania, respectively did not fit under any of the currently eight identified races though they behave like races 1 and 2. In an earlier study, we reported similar identification of isolates that lack *Ptr ToxA* gene but are equally virulent like race 1 from Arkansas, USA

(Ali et al., 2010). Similarly, 50% of *P. tritici-repentis* isolates were devoid of *Ptr ToxA* gene but behaved like race 1 and 2 from Russia, a Balkan state neighboring country (Mironenko et al., 2015). It is likely that wheat cultivars grown in the region, especially in Lithuania may not require Ptr ToxA sensitivity for susceptibility to tan spot. This similar behavior (insensitive to Ptr ToxA and but susceptible to tan spot) has also been reported in wheat cultivars in many independent studies (Ali et al., 2002; Ali et al., 2010; Noriel et al., 2011; Oliver et al., 2008). Our results show prevalence of diverse population of *P. tritici-repentis*, with race 1 (contains Ptr ToxA and Ptr ToxC) being most abundant in the surveyed area. Further, fungal isolates lacking *Ptr ToxA* gene but behave either like race 1 or 2 were identified suggesting the need further investigate if these isolates produce new toxins or use different arsenals to be pathogenic on wheat. Breeding of tan spot resistant wheat cultivars against race 1 should provide sufficient resistance to minimize losses occurring due to tan spot in the region. However, tan spot resistant germplasm should also be tested against isolates lacking in *Ptr ToxA* gene but act like races 1 or 2 to be sure if they may carry different toxins from race 1 and hence make race 1 resistant cultivars vulnerable to tan spot. Also, the fungal pathogen should be monitored for any virulence change periodically.

Our results further show that *Ptr ToxA* and *Ptr ToxB* genes specific markers can be useful in distinguishing isolates that carry these two genes. However, in the absence of availability of molecular markers for all toxins and the possibility of unknown toxins, characterization of races carrying these toxins by molecular approaches is not realistic. Characterization of *P. tritici-repentis* races using tan spot wheat differential set for now

is the most robust method for exploring variability in the pathogen population until precise molecular markers for all toxins are developed.

ACKNOWLEDGEMENTS

We like to thank Mr. Richard Gappert for his help in greenhouse and lab work. The South Dakota Wheat Commission and USDA NIFA through SDSU Agricultural Experiment Station (AES) provided financial support for this research.

LITERATURE CITED

Aboudkhaddour, R., Kelly, T. T., and Stephen, E. S. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. *Can J. Plant Pathol*. 35:256-268 http://dx.doi.org/10.1094/PDIS-03-16-0295-PDN.

Ali, S. and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses. *Plant Dis.* 87:418-422.

Ali, S. and Francl, L. J. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. *Plant Dis.* 85:580–584.

Ali, S., and Francl, L. J. 2002. Race structure of *Pyrenophora tritici-repentis* isolates obtained from wheat in South America. *Plant Protection Science* 38:302-304.

Ali, S., Sarova, J. and Waukulinsky, W. 2004. *Pyrenophora tritici-repentis* races in the Czech Republic and Poland. *Phytopathology* 93:(Suppl.), S3.

Ali, S., Singh, P. K., McMullen, M. P., Mergoum, M., and Adhikari, B. T. 2008. Resistance to multiple leaf spot diseases in wheat. *Euphytica* 159:167-179.

Ali, S., Gurung, S. and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Dis.* 94:229–235.

Andrie, R. M., Pandelova, I. and Ciuffetti, L. M. 2007. A combination of phenotypic and genotypic characterization *Pyrenophora tritici-repentis* race identification. *Phytopathology* 97:694-701

Andrie, R. M., Schoch, C. L., Hedges, R., Spatafora, J. W., and Ciuffetti, L. M. 2008. ToxB, a host-selective toxin gene from *Pyrenophora triticirepentis*, is present in the genome of

sister-species P. bromi and other members of the Ascomycota. *Fungal Genet. Biol.* 45:363-377.

Benslimane, H., Lamari, L., Benbelkacem, A., Sayoud, R. and Bouznad, Z. 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identification of a new virulence type. *Phytopathol. Mediterr*. 50:203−211.

Beruta, B. and Ilze, P. 2011. A review of tan spot research in Baltic countries. *Zemdirbyste=Agriculture* 98: 3-10.

Ciuffetti, L. M., and Tuori, R. P. 1999. Advances in the characterization of the *Pyrenophora tritici-repentis*-wheat interaction. *Phytopathology* 89:444–449.

De Wolf, E. D., Effertz, R. J., Ali, S. and Francl, L. J. 1998. Vistas of tan spot research. *Can. J Plant Pathol.* 20:349–370.

Effertz, R. J., Meinhardt, S. W., Anderson, J. A., Jordahl, J. G. and Francl, L. J. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. *Phytopathology* 92:527–533

Engle, J. S., Madden, L. V. and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology* 96:1355-1362.

Francl, L. J. 1997. Local and mesoscale dis- persal of *Pyrenophora tritici-repentis* conidia. *Can. J. Plant Pathol*. 19:247-255.

Friesen, T. L., Ali S., Kianian, S., Francl L. J., and Rasmussen, J. B. 2003. Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. *Phytopathology* 93:397–401

Gamba, F. M., Strelkov, S. and Lamari, L. 2012. Virulence of *Pyrenophora tritici-repentis* in the Southern Cone Region of South America. *Can. J. Plant Patho*l. 34:545–550.

Hosford, R. M. 1982. Tan spot - developing knowledge 1902-1981, virulent races and wheat differentials, methodology, rating systems, other leaf diseases, literature. In R. M. Hosford (Ed.), Tan Spot of Wheat and Related Disease Workshop (pp. 1-20). Fargo, ND: North Dakota Agric. Exp. Station.

Jordahl, J. G., and Francl, L. J. 1992. Increase and storage of cultures of *Pyrenophora tritici-repentis*. Page 109 in: Advances in Tan Spot Research. L. J. Francl, J. M. Krupinsky, and M. P. McMullen, eds. North Dakota Agric. Exp. Stn., Fargo.

Krupinsky, J. M. 1992. Grass hosts of *Pyrenophora tritici repentis. Plant Dis.* 76: 92–95.

Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. *Can. J. Plant Pathol*. 11:49-56.

Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. *Phytopathology* 93:391 396.

Lamari, L., Sayoud, R., Boulif, M. and Bernier, C. C. 1995. Identification a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. *Can. J. Plant Pathol.* 17:312-318.

Liatukas, Z., Ruzgas, V. and Razbadauskienė, A. 2012. Winter wheat cultivars Kovas DS, Zunda DS., Vikaras, DS., Kaskada DS for high input farming: Development and characterization. *Zemdirbyste=Agriculture* 99:255-264.

Lepoint, P., Renard, M.-E., Legrève, A., Duveiller, E. and Maraite, H. 2010. Genetic diversity of the mating type and toxin production genes in *Pyrenophora tritici-repentis*. *Phytopathology* 100:474-483.

Luz da, W. C. and Hosford, R. M. 1980. Twelve *Pyrenophora trichostoma* races for virulence to wheat in the central plains of North America. *Phytopathology* 70:1193-1196.

Mikhalova, L. A., Mironenko, N. V. and Kovalenko, N. M. 2014. Populations of *Pyrenophora tritici-repentis* in the North Caucasus and North-West Russia: The racial composition and dynamics of virulence. *Mikol. Fitopatol.* 48:393–400.

Mironenko, N. V., Baranova, O. A., Kovalenko, N. M. and Mikhalova, L. A. 2015. Frequency of ToxA gene in North Caucasian and North-West Russian populations of *Pyrenophora tritici-repentis*. *Mikol. Fitopatol.* 49:325–329.

Misra, A. P. and Singh, R. A. 1972. Pathogenic differences among three isolates of *Helminthosporium tritici-repentis* and the performance of wheat varieties against them. *Indian Phytopathol*. 25:350-353.

Moreno, M. V., Stenglein, S. A., Ballati, P. A. and Perello, A. E. 2008. Pathogenic and molecular variability among isolates of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat in Argentina. *Eur. J. Plant Pathol*. 122:239-252.

Noriel, A. J., Xiaochum, S. Willimam, B. and Guihua, B. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. *Crop Science* 51: 1059-1067.

Ronis, A., Semaškiene, R., Dabkevicius, Z. and Liatukas, Z. 2009. Influence of leaf diseases on grain yield and yield components in winter wheat. *J. Plant Protect.* 49:151-157.

Sarova, J., Hanzalova, A. and Bartos, B. 2005. Races of *Pyrenophora tritici-repentis* in Czech Republic. *Acta Agrobotanica* 58:73-78.

Schilder, A. M. C., and Bergstrom, G. C. 1990. Variation in virulence within the population of *Pyrenophora tritici-repentis* in New York. *Phytopathology* 80:84-90.

Shabeer, A., and Bockus, W. W. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Dis.* 72:599-602.

Sierotzki, H., Frey, R., Wullschleger, J., Palermo, S., Karlin, S., Godwin, J., and Gisi, U. 2007. Cytochrome *b* gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. *Pest Management Scien*ce 63:225-233.

Singh, P. K., and Hughes, G. R. 2006. Genetic Similarity among isolates of *Pyrenophora tritici-repentis,* causal agent of tan spot of wheat. *J. Phytopathol*. 154:178-184.

Singh, P. K., Mergoum, M., Ali, S., Adhikari, T. B., Elias, E. M., Anderson, J. A., Glover, K. D. and Berzonsky, W. A. 2006. Evaluation of elite wheat germ plasm for resistance to tan spot. *Plant Dis.* 90:1320-1325.

USDA FAS 2016. Gain report. http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Grain%20and%20Feed%20Annual%20-%20Lithuania%202016_Warsaw_Lithuania_4-14-2016.pdf

CHAPTER 4

TITLE: REACTION OF GLOBAL COLLECTION OF RYE (*SECALE CEREALE* L.) TO TAN SPOT AND PYRENOPHORA *TRITICI-REPENTIS* RACES IN SOUTH DAKOTA

Abdullah, S., Sehgal, S. K., Glover, K. D. and Ali, S. 2017. 2017. Reaction of global collection of rye (*Secale cereale* L.) to tan spot and *Pyrenophora tritici-repentis* races in South Dakota. *Plant Pathol. J.* (**Accepted 14-Mar-2017).**

ABSTRACT

Rye (*Secale cereale* L.) serves as an alternative host of *Pyrenophora tritici-repentis* (*Ptr*) the cause of tan spot on wheat. Rye is cultivated as a forage or cover crop and overlaps with a significant portion of wheat acreage in the U.S. Northern Great Plains, however, it is not known whether the rye crop influences the evolution of *Ptr* races. We evaluated a global collection of 211 rye accessions against tan spot and assessed the diversity in *Ptr* population on rye in South Dakota. All the rye genotypes were inoculated with *Ptr* races 1 and 5, and infiltrated with Ptr ToxA and Ptr ToxB, at seedling stage. We observed 21% of the genotypes exhibited susceptibility to race 1, whereas, 39% were susceptible to race 5. All 211 accessions were insensitive to both the Ptr toxins. It indicates that though rye exhibits diversity in reaction to tan spot, it lacks Ptr ToxA and ToxB sensitivity genes. This suggests that unknown toxins or other factors can lead to *Ptr* establishment in rye. We characterized the race structure of 103 *Ptr* isolates recovered from rye in South Dakota. Only 22% of the isolates amplified *Ptr ToxA* gene and were identified as race 1 based on their phenotypic reaction on the differential set. The remaining 80 isolates were noted to be race 4. Our results show that races 1 and 4 are prevalent on rye in South Dakota with a higher frequency of race 4, suggesting a minimal role of rye in the disease epidemiology.

Key words: *Secale cereale*, host-selective toxins (Ptr ToxA and Ptr ToxB), *Ptr* races, tan spot, rye

INTRODUCTION

The fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph = *Drechslera tritici-repentis* (Died.) Shoem.) is an important foliar pathogen of wheat (*Triticum aestivum* L.), and causes tan spot throughout major wheat-growing countries (De Wolf et al., 1998). The fungus has a broad host-range and can infect barley, oat, rye and many non-cereal grasses (Ali and Francl, 2003; Hosford, 1971; Krupinsky, 1992; Sprague, 1950). The fungus produces oval-shaped tan necrotic spots on the leaf surrounded by a chlorotic halo with a pinhead size black spot in the center on susceptible wheat and alternative hosts genotypes (Hosford, 1971; Krupinsky, 1992). Eight races have been identified in the fungal population based on an isolate's ability to produce necrosis and/or chlorosis symptoms on appropriate tan spot differential genotypes (Lamari et al., 2003). The fungus is also known to produce three host-selective toxins, Ptr ToxA, Ptr ToxB, and Ptr ToxC, which are associated with development necrosis and chlorosis symptoms (Faris et al., 2013; Lamari et al., 2003). Of these 8 races, 1 through 5 have been reported in North America (Ali and Francl, 2003; Lamari et al., 2003). The most prevalent race on wheat worldwide is race 1, whereas race 4 is the least observed (Aboudkhaddour et al., 2013; Ali and Francl, 2002; Ali and Francl, 2003; Lamari et al., 2003). In contrast, race 4 was the most prevalent race observed on its alternative hosts such as non-cereal grasses (Ali and Francl, 2003).

Knowledge of genetic variation in the pathogen virulence and the host plant is crucial in the development of effective and durable disease management strategies especially breeding for cultivars with durable disease resistance. Variability of virulence

and aggressiveness has been reported in the *Pyrenophora tritici-repentis* (*Ptr*) isolates recovered from various alternative hosts such as Altai wild rye, Basin wild rye, and Russian wild rye (Ali and Francl, 2003; Krupinsky, 1992). However, the prevalence of *Ptr* population on rye has not been studied. Further, diversity to tan spot reaction in rye has not been explored. Alternative host plants can play a significant role in pathogen diversity and disease epidemic, especially when they are cultivated in the vicinity of the economically important host crops (Burdon, 1993). In contrast, alternative hosts can also be a part of the solution by contributing resistance genes to pests and diseases (Dinoor, 1974). Rye (*Secale cereale* subsp. *cereale*) is one of the most important cereal crops worldwide that is primarily grown as forage for livestock, cover crop for green manure, or as a food product for use in bread. (Bushuk, 2001). Moreover, rye has played a significant role in modern wheat improvement through its contribution of resistance genes for combating leaf rust, stem rust, stripe rust, powdery mildew, BYDV and insect resistance to Hessian fly, Russian wheat aphids, and Green bug (Rabinovich, 1998; Saulescu et al., 2011; Zhang et al., 2001) as examples.

In the NGP, rye is most often grown as a green manure and a rotational crop that is often planted adjacent to wheat fields. This can potentially play a role in *Ptr* virulence variation and evolution; harbor completely different pathogen population; and/or serve as an additional source of inoculum for tan spot development on wheat. The objectives of this study were: i) to evaluate a global collection of rye genotypes for their reaction to tan spot to determine if rye could be exploited as a source of resistance to *Ptr*; ii) to determine if rye could be playing any role in the *Ptr* diversity in South Dakota. The study

will contribute to our understanding of the genetic diversity for tan spot resistance in rye that could be exploited by both wheat and rye breeders. Further, our study underpins the virulence pattern of *Ptr* on rye that could guide in tan spot management strategies.

MATERIAL AND METHODS

Evaluation of rye genotypes to their reaction tan spot

Two hundred and eleven rye genotypes from 54 countries representing Asia, Africa, Europe, North America and South America, (Figure 4.1) were evaluated for their reaction to tan spot using *Ptr* races 1 and 5 and their host selective toxins, Ptr ToxA and Ptr ToxB, respectively. Due to lack of seed, only 171 out of 211 genotypes were evaluated against race 5. The seed of all genotypes were obtained from USDA-ARS National Small Grains Collection, Aberdeen, Idaho. Additionally, eight rye cultivars, Rymin, Dreb 15, ND 5, Fredrick, ND Dylan, Muskter, Dacold, and Spooner grown in the region was included in this study. All 211 genotypes, except four [*Secale varilovii* (n=1); *S. cereale* subsp. ancestrale (1); *S. strictum* (2)], were of *S. cereale* subsp. cereale.

Figure 4.1. Origin of 211 rye genotypes evaluated in this study.

Race 1 being most prevalent race worldwide and race 5 having been observed in the NGP, were selected for this study (Ali et al., 1999; Abdullah et al., 2016). SD13-101 isolate of race 1 and SD13-103 isolate of race 5 were used to evaluate two-week old seedlings of all rye genotypes grown in containers (5 x 23 cm) filled with Sunshine Mix (770 Silver Street, Agawam, MA). The two isolates used in this study were recovered from wheat grown in South Dakota (Abdullah et al., 2016). We planted three seedlings per container (per replication) and a total of nine seedlings (3 replications) of each genotype were tested. The seedlings were watered and fertilized as needed throughout the experimentation. Experiments were conducted in the greenhouse at South Dakota State University with an average temperature of 21^{o}C and 18^{o}C during the day and night, respectively, and a 16-h photoperiod was imposed. All 211 and 171 genotypes were evaluated for their reaction to race 1 and race 5, respectively by following the

procedure described by Ali and Francl (2001). In brief, two-week old seedlings of all rye genotypes were inoculated individually with race 1 and race 5 by spraying with a spore suspension adjusted to 2500 spores/ml until runoff with a hand-held sprayer (Preval Sprayer, Chicago Aerosol, 1300 E. North Street, Coal City, IL). Inoculated seedlings were placed in a humidity chamber set at 100% humidity, using a humidity controller set to mist 16 seconds every 5 minutes for 24 hours. Thereafter, seedlings were placed on a greenhouse bench for seven days until plants were scored for symptom development using a 1-5 disease rating scale where 1-2 = resistant to moderately resistant; 3 - 5 = moderately susceptible to susceptible (Lamari and Bernier, 1989). To confirm the success of the inoculation procedure, the wheat differential lines Glenlea (susceptible to race 1), 6B365 (susceptible to race 1), 6B662 (susceptible to race 5), and Salmouni (resistant to race 1 and race 5) were included in the experiment as checks.

Reaction of rye genotypes to Ptr ToxA and Ptr ToxB

All 211- and 171-rye genotypes tested for against race 1 and race 5 were also screened for their reaction to Ptr ToxA and Ptr ToxB, respectively. Seedlings of all genotypes were raised in plastic containers. Three fully expanded second leaves of each genotype were infiltrated individually with purified Ptr ToxA at 10 µg/ml and Ptr ToxB culture filtrates about 10 µl/leaf using a needleless syringe as described by Effertz et al. (1996). The infiltrated leaf area was marked with a permanent non-toxic black marker. Infiltrated leaves were rated for sensitivity three days' post-infiltration of Ptr ToxA for presence/absence of necrosis and five days post-infiltration of Ptr ToxB for presence/absence of chlorosis in the infiltrated area. Purified Ptr ToxA and Ptr ToxB

culture filtrates were kindly provided by Dr. S. Mienhardt, Department of Plant Pathology, North Dakota State University, Fargo, ND 58102 and Dr. T. Friesen, USDA-ARS, Fargo, ND 58102, respectively.

Recovery of *Ptr* isolates from rye leaves

Diseased leaf samples exhibiting tan spot like symptoms were randomly collected from commercial fields and SDSU experimental field plots during 2013 and 2014 growing seasons. Leaf samples were collected from 4 locations in 2013 and five locations in 2014. The samples were collected when the crop was at late milk to soft dough stage. *Ptr* isolates were recovered from the collected leaf samples by following the procedure described by Ali and Francl (2001). Thereafter, leaves of each sample were cut into 1.5 to 2 cm long segments and surface-disinfected with 5% bleach (NaClO). Forty-fifty randomly selected leaf segments from each sample were placed in a petri dish (9 cm dia) containing three layers of moist Whatman #1 filter paper. Plates were incubated for 24 hours under light at room temperature (21-22^{o}C) and 24 hours in dark at 16^{o}C to induce conidial formation. The incubated leaf segments were examined with a stereoscope, and single conidia were collected using a flamed steel needle and then placed on a fresh V8-PDA (150 ml of V8 juice, 10 g of PDA, 10 g of Difco agar, 3 g of CaCO3, and 850 ml of distilled water) plate (Lamari and Bernier, 1989). In total, 103 isolates were recovered and stored at -20^{o}C until characterized for their race structure. (Appendix Table 4.2).

Genotyping of *Ptr* isolates from rye for *Ptr ToxA* and *Ptr ToxB* genes

DNA of all 103 *Ptr* isolates recovered from rye was isolated by growing them individually on V8-PDA in 9 cm petri dishes for 5 days. Mycelia were scraped from the agar surface using a flamed scalpel and placed in a 2 ml microcentrifuge tube. The mycelia were then dried overnight in a water bath at 37°C and were ground into a fine powder using a first prep machine Retsch MM 301 (Retsch., Clifton, NJ). DNA was extracted from mycelia of each isolate by following the procedure of Moreno et al. (2008). The DNA concentration was adjusted to 25 ng/µl using a Nanodrop Spectrophotometer (Counterpane Inc. Tacoma, WA) and run in a 0.8% agarose gel to verify quality. The *Ptr* isolates were genotyped for *Ptr ToxA* and *Ptr ToxB* genes by using the *Ptr ToxA* and *Ptr ToxB* specific primers developed by Andrie et al. (2007). Conformity of the isolates was determined by using two *Ptr* mating type genes (*MAT1-1* and *MAT1-2*) specific primers suggested by Lepoint et al. (2010). PCRs for specific markers were performed in 20 µl volume including; 2 µl genomic DNA (25 ng/µl, 0.8 µl of each primer (10mM), 0.5 µl dNTP (200 µM), 2 µl 10 x thermophol buffer, 0.2 µl 10 U/ml *Taq* Polymerase and 13.7 µl of molecular biology grade water. PCR reaction for individual primers was conducted in a S-1000 thermal cycler (BioRad, Hercules, CA 94547) using amplification steps of 94°C for 3 min, followed by 30 cycles of 94°C for 45 seconds, 55°C for 30 seconds and 72°C for 1 min and a final extension of 72°C for 7 min. We pooled PCR products from housekeeping genes (*MAT1-1* or *MAT1-2) with Ptr ToxA* or *Ptr ToxB* specific PCR products as a positive amplification control for each isolate. The PCR

products were electrophoresed on 1.5% agarose gels and scored with reference to 1 Kb ladder (New England Biolabs, Ipswich, MA 01938).

Phenotyping of *Ptr* isolates

For race characterization of all 103 isolates, two-week old seedlings of the tan spot differential genotypes Glenlea, 6B365, 6B662, and Salamouni were inoculated individually with each isolate using spore suspensions (2500 spores/ml). Inoculum preparation and inoculation were performed as described by Ali and Francl (2001). Inoculated seedlings with each isolate were rated for necrosis and chlorosis symptom development and the isolates were grouped under appropriate race (Lamari et al., 2003). (Appendix Table 4.2).

RESULTS AND DISCUSSION

Reaction of rye genotypes to tan spot (race 1) and Ptr ToxA

All 211 rye genotypes evaluated against tan spot responded differentially to *Ptr* race 1 by exhibiting reactions that ranged from moderately susceptible (MS) to susceptible (S) and moderately resistant (MR) to resistant (R). Of the eight rye cultivars screened only ND5 exhibited a moderately susceptible (lesion type 3) reaction to race 1 while others showed a moderately resistant reaction. Nearly 21% (n= 44) of the genotypes showed susceptibility (lesion type 3-5) to race 1 (Table 4.1 and Appendix Table 4.1) while the other 79% (n= 167) exhibited resistance (lesion type 1-2). The ratio of susceptible to resistant genotypes against race 1 was about 1:3 in genotypes from Africa (2 susceptible and 6 resistant), Europe (22 susceptible and 67 resistant), North America (10 susceptible and 32 resistant), and South America (4 susceptible and 12 resistant) (Table 4.1); whereas, about 1:7 (6 susceptible and 46 resistant) in genotypes from Asia suggesting higher prevalence of resistant germplasm or varieties in Asia as compared to other continents. All 211 rye accessions and eight cultivars from NGP, when infiltrated with Ptr ToxA exhibited insensitivity as they did not develop necrosis in the toxin infiltrated areas (Figure 4.2). As expected, tan spot differentials Glenlea and 6B365 on inoculation with race 1 developed necrosis and chlorosis symptoms, respectively; whereas, 6B662 and Salamouni remained symptomless. Also, Glenlea exhibited sensitivity (developed necrosis) and Salamouni insensitivity (no necrosis) in the toxin-infiltrated area, validating the inoculation procedure and conformity of race 1 and the toxin viability.

We observed susceptible to resistant reactions on a large number of rye genotypes to *Ptr* (tan spot) race 1, suggesting variability for resistance to tan spot exists in rye. Our results with a global collection of 211 rye genotypes validate previous studies where a small number of rye accessions were screened and differential response to tan spot was observed (Cox et al., 2005; Hosford, 1971; Oliver et al., 2008a; Postnikova and Khasvova, 1998). Cox et al. (2005) evaluated 10 perennial grass species, including two perennial rye genotypes for tan spot using *Ptr* ace 1 and found them resistant, similar to the resistant wheat cultivar 'Karl 92' used as a check in their study. Similarly, Oliver et al. (2008a) evaluated 199 wheat-alien species derivatives for tan spot and Stagonospora nodorum blotch (SNB) resistance and found some of the derivatives from rye were resistant to both diseases and concluded that resistance to tan spot in these wheat alien species derivatives came from the rye. In contrast to these two studies, rye was found equally susceptible as the wheat genotype used as tan spot susceptible check in two independent studies for *Ptr* host range (Hosford, 1971; Postnikova and Khasvova, 1998). However, a single genotype was evaluated in each study to confirm whether rye could be an alternative host of *Ptr*. It is not surprising to observe diverse reactions to tan spot in rye, similar observations have been reported in other alternative hosts of *Ptr* that exhibited from susceptible to resistant reactions (Ali and Francl, 2001; Ali et al., 2015; Krupinsky, 1982 and 1992). Both resistant and susceptible responses to race 1 were observed among genotypes from all six continents; however, a higher ratio of resistant to susceptible genotypes was observed in all continents (1 susceptibe:3 resistant) except in Asia (1:7). This could be due to less selection pressure on the pathogen surviving on

alternative hosts. Interestingly, all 211 genotypes and 8 cultivars evaluated irrespective of the fungal susceptibility or resistance showed insensitivity to Ptr ToxA, indicating a lack of *Tsn1* (Ptr ToxA sensitivity gene). This suggests that Ptr ToxA may not play a significant role in disease development on rye and that perhaps other factors such as unknown toxins may be responsible for tan spot on rye. Similar observations have been reported (Ali et al., 2010; Faris and Friesen, 2005; Noriel et al., 2011; Oliver et al., 2008b; Zhang and Jin, 1998) where wheat and its wild relatives showed susceptibility to Ptr ToxA producing races 1 and 2 but remained insensitive to Ptr ToxA. Our earlier studies along with others have also suggested the possibile involvement of some other potential toxins or mechanisms for compatible tan spot-wheat host-pathogen interaction (Ali et al., 2010; Faris and Friesen, 2005; Noriel et al., 2011; Oliver et al., 2008b; Zhang and Jin, 1998). Very recently, Virdi et al. (2016) studied the role of Ptr ToxA in tan spot development on durum wheat and concluded that role of Ptr ToxA was insignificant. Additionally, the fungal isolates lacking the Ptr ToxA gene, but virulent on both Ptr ToxA sensitive and insensitive wheat genotypes indicates a minimal role of Ptr ToxA, however, the role of Ptr ToxA in tan spot development may be genotype dependent. Alternatively, it is possible that Ptr ToxA lacking isolates may carry additional virulence factors causing disease induction (Ali et al., 2010).

Table 4.1. Reaction of 211 rye genotypes from six continents to *P. tritici-repentis* race 1 and PtrToxA

Continent	Genotypes	Susceptible (%)	Resistant (%)	Ptr ToxA insensitive (%)
Africa	8	2 (25.0)	6 (75.0)	8 (100)
Asia	52	6 (11.5)	46 (88.4)	52 (100)
Australia	4	0 (0)	4 (100.0)	4 (100)
Europe	89	22 (24.7)	67 (75.2)	89 (100)
N. America	42	10 (23.8)	32 (76.2)	42 (100)
S. America	16	4 (25.0)	12 (75.0)	12 (100)
Total	**211**	**44 (20.8)**	**167 (79.1)**	**211 (100)**

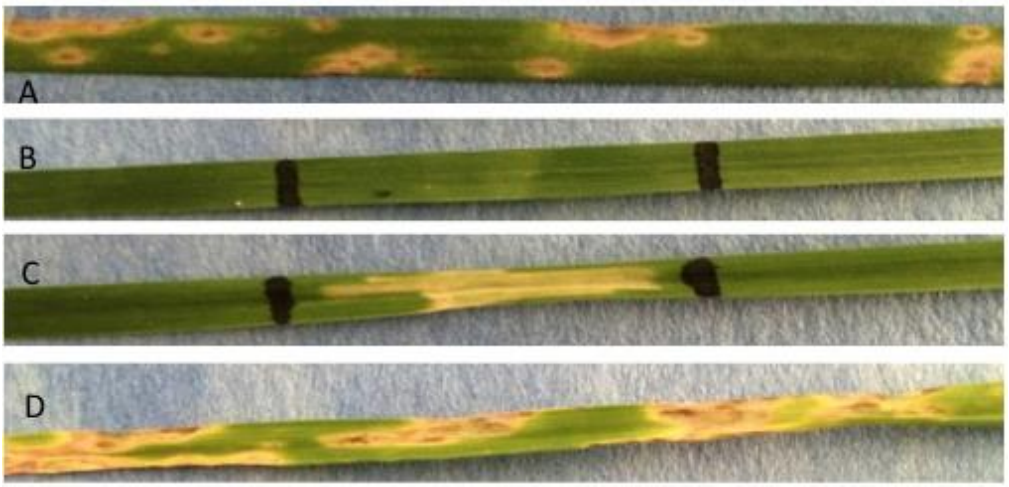

Figure 4.2. Reaction of rye genotype to *P. tritici-repentis* race 1 and Ptr ToxA (Top-bottom). A) Rye genotype (PI446514) inoculated with race 1 (necrosis), B) rye genotype (PI446514) infiltrated with Ptr ToxA (no necrosis), C) wheat genotype Glenlea infiltrated with Ptr ToxA (necrosis), and D) Glenlea inoculated with race 1 (necrosis).

Reaction of rye genotypes to tan spot (race 5) and Ptr ToxB

We screened 171 rye genotypes for their reaction to tan spot (race 5) and observed a range of reactions from moderately susceptible to susceptible and moderately resistant to resistant (Table 4.2; Appendix Table 4.1). Nearly 38.6% (n=66) of the genotypes exhibited susceptibility while the other 61.4% (n=105) genotypes were

resistant to race 5 (Table 4.2; Appendix Table 4.1). All the 66 genotypes that exhibited susceptibility to race 5 did not exhibit sensitivity to Ptr ToxB (Figure 4.3). Tan spot susceptible and resistant rye genotypes were observed from all continents except Australia (Table 4.2) but the percentage of susceptible to resistant genotypes varied among continents. Of the genotypes with European origin, 47.3% (n=36) were susceptible and 52.7% (n=40) were resistant to race 5, whereas, among genotypes from Asia, only 25.0% (n=11) were susceptible and nearly 75.0% (n=33) were resistant to race 5 (Table 4.2). Four (Derb 15, Fredrick, Muskter, and Spooner) of the eight cultivars grown in NGP showed susceptibility with lesion type ranging from 3-5, whereas the other four cultivars were rated as resistant (Appendix Table 4.1). Overall, the percent of susceptible genotypes to race 5 was lower (38.6%) as compared to resistant genotypes (61.4%) across all the continents although European genotypes were most susceptible (47.3%). Higher level susceptibility in European rye germplasm to race 5, is expected because Europe (Belarus, Germany, Poland, Ukraine, and western Russia) produces 80% of the rye grown in the world (Bushuk, 2001). Most likely this originates from the use of race 5 susceptible germplasm in the breeding programs. Similar observations were reported by Lamari et al. 2005 in wheat in Canada when they studied the reason (s) of increased susceptibility in Canadian wheat cultivars to race 5 in the 1960's. They were able to trace it back to one susceptible of the parent "Kanred Winter" of cultivar Thatcher that had been heavily utilized in developing high yielding and stem rust resistance cultivars in Canada and North Dakota, USA in 1960's. Further 14 of 211 rye genotypes exhibited susceptibility to both race 1 and race 5 (Table 4.3). The presence of

genotypes susceptible to both races may help in the survival of race 1 and race 5 on rye and may lead to the evolution of new races. As expected, the wheat differential genotype 6B662 inoculated with race 5 and infiltrated with Ptr ToxB toxin developed chlorosis to both spore inoculations and the toxin infiltration, while Glenlea, 6B365, and Salamouni exhibited resistance to race 5 and insensitivity to Ptr ToxB. This verified the successful inoculation process, and confirmed race 5 isolate and Ptr ToxB viability. The presence of race 5 susceptibility in about 1/3 of the rye genotypes from across the globe and half of the commercially grown cultivars in the region is very surprising because race 5 was not observed until 1995, when it was first reported from Algeria (Lamari et al., 1995) and thereafter in Asia, and North America (Abdullah et al., 2016; Ali et al., 1999; Lamari et al., 2003). Widespread susceptibility to race 5 in rye genotypes suggests that race 5 has been present much longer than 22 years and perhaps earlier than race 1 in most of wheat growing countries. In contrast, susceptibility to race 5 could be due to use of rye germplasm with good agronomic characters but susceptible to tan spot race 5 in the cultivars development as it has been observed in wheat cultivars susceptible to race 5 developed in the 1960's (Lamari et al. 2005), even though the race 5 had not been reported until recently in North America (Ali et al., 1999; Lamari et al., 2003). Based on our results, it can be hypothesized that rye could be a contributor to race 5 resistance and/or susceptibility, especially in Ptr ToxB insensitive wheat genotypes where rye has been used in wheat improvement. Triticale serves as a bridge between rye and the modern wheat. Evaluation of triticale germplasm against multiple races of

Ptr may provide additional insight into the role rye has played in susceptibility or resistance to tan spot race 5.

Table 4.2. Reaction of 171 rye genotypes from six continents to *P. tritici-repentis* race 5 and Ptr ToxB

Continent	Genotypes	Susceptible (%)	Resistant (%)	Ptr ToxB Insenstive (%)
Africa	7	2 (28.5)	5 (71.4)	9 (100)
Asia	44	11 (25.0)	33 (75.0)	44 (100)
Australia	4	0 (0)	4 (100)	4 (100)
Europe	76	36 (47.3)	40 (52.6)	76 (100)
N. America	29	11 (37.9)	18 (62.0)	29 (100)
S. America	11	6 (54.5)	5 (45.4)	11 (100)
Total	**171**	**66 (38.5)**	**105 (61.4)**	**171 (100)**

Figure 4.3. Reaction of rye genotypes to *P. tritici-repentis* race 5 and Ptr ToxB (Top to Bottom). A) Wheat genotype 6B662, B) rye cultivars 'Fredric' (chlorosis -susceptible), C), 'Muskater' (moderately susceptible), D) 'ND 5' (resistant), E) wheat genotype Salamouni (resistant) inoculated with *P. tritici-repentis* race 5, F) wheat genotype 6B662, G) rye cultivar Fredric, and H) wheat genotype Salamouni infiltrated with Ptr ToxB

Table 4.3. Fourteen rye (*Secale cereale subsp. cereale*) genotypes susceptible to tan spot against *P. tritici-repentis* race 1 and race 5

Country	Accession	Race 5		Race 1	
		Lesion type	Reaction	Lesion type	Reaction
Austria	PI 534960	4	S	4	MS
Belgium	PI 534970	3	MS	3	MS
Chile	PI 436171	4	S	3.5	MS
China	PI 452133	4	S	4	S
Estonia	PI 446514	4	S	4	S
France	PI 315957	3	MS	3.5	MS
Germany	PI 290435	3	MS	1	MS
Lithuania	PI 446123	5	S	3	MS
Montenegro	PI 344980	3	MS	3.5	MS
Morocco	PI 525205	4	S	4	S
Portugal	PI 535094	4	S	3	MS
Romania	PI 446245	3	MS	3	MS
South Africa	PI 330407	4	S	3.7	MS
Sweden	PI 368157	4	S	3.75	MS

S = susceptible; MS = moderately susceptible; Plants were rated on lesion type 1-5 scale wherein 1-2 are resistant and 3-5 are moderately susceptible to susceptible (Lamari and Bernier 1989).

Recovery of *P. tritici-repentis* isolates, their genotyping for *Ptr ToxA* and *Ptr ToxB* genes and phenotyping for race characterization

All rye leaf samples collected from nine SD locations were analyzed for harboring *Ptr* and the frequency of recovery ranged from 1 to 20% on the plated leaf segments. In 2013, we recovered 20 *Ptr* isolates, whereas 83 isolates were recovered in 2014. All 103 isolates were evaluated for the presence of *Ptr ToxA* and *Ptr ToxB* genes, however, only 23 isolates amplified *Ptr ToxA* and the other 80 isolates lacked in both *Ptr ToxA* and *Ptr ToxB* (Table 4.4). As expected the positive controls (race 1 and race 5) amplified bands of 585bp and 295bp corresponding to the *Ptr ToxA* and *Ptr ToxB* respectively, thus validating the primers and the PCR procedure (Figure 4.4). The genotypic data suggests that the isolates that harbor *Ptr ToxA* could possibly be race 1 or race 2. The other 80

isolates lacking in both *Ptr ToxA* and *Ptr ToxB* could potentially be race 3 or race 4 as neither of these races carry the genes. However, due to unavailability of a *Ptr ToxC* gene-specific DNA marker, it is not possible to successfully discriminate these isolates as race 3 or race 4. Presently the only method to characterize such isolates is through phenotyping with the tan spot differential set. Further, isolates that lack in both toxins genes may not be even *Ptr*, however, we confirmed the identity of all 103 isolates through amplification of *Ptr* specific housekeeping genes and eliminated this possibility.

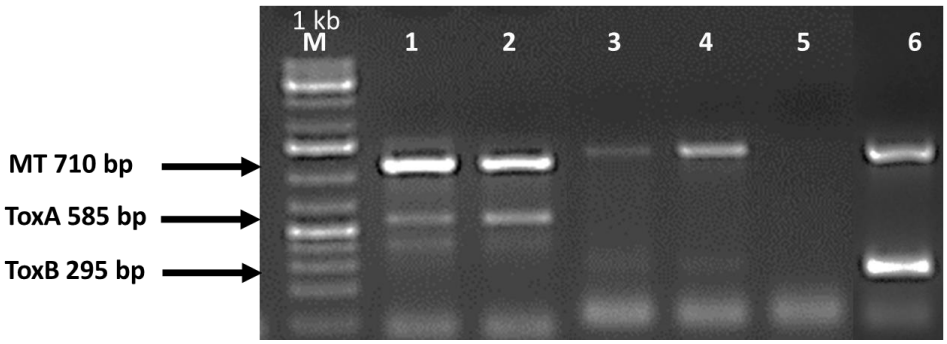

Figure 4.4. Gel picture of the *Ptr* isolates showing the presence of *ToxA*, *ToxB* and Mating type (*MAT1-1*) gene of *Ptr* as an internal control. Lane 1: *ToxA* (from wheat race 1 (SD13-101) positive control); Lane 2: *ToxA* (from rye race 1 (13-3-P1.1)); Lane 3: Race 4 (isolates from wheat, 13-103-P4.30) lacking *ToxA* and *ToxB*; Lane 4: Race 4 (from rye, 14-40-P6) lacking *ToxA* and *ToxB*; Lane 5: Water as negative control; Lane 6: *ToxB* (from wheat race 5 (SD13-103) positive control).

We phenotyped 23 isolates that harbored *Ptr ToxA* on wheat differentials and all isolates were grouped as race 1 because they incited necrosis on wheat genotype Glenlea, chlorosis on 6B365, and no symptoms on 6B662 and Salamouni. The 80 isolates that lacked both *Ptr ToxA* and *Ptr ToxB* did not produce necrosis and/or chlorosis on any

of the four differential genotypes and were therefore characterized as race 4 (Table 4.4).

Prevalence of *Ptr* race 4 on rye is not surprising, as this had been observed in abundance on other alternative host plant species (Ali and Francl, 2001, 2003). However, the reasons for the existence of race 4 in high frequency are not clear. Race 4 has never been tested for virulence on a large number of wheat or alternative host genotypes. The colony growth of race 4 from rye was green olivecious in color and low in spore production (data not shown) as compared to race 1 isolates recovered from rye and wheat (Figure 4.5). Rye and wheat are generally cultivated in the same niche during the cropping season in the NGP. However, to our knowledge, rye has never been studied in the past for its role, if any, in the diversity of *Ptr* races as has been done on wheat in the region and anywhere else.

Table 4.4. Genotypic and phenotypic characterization of *P. tritici-repentis* isolates recovered from rye in 2013 and 2014

Year	Number of isolates tested	Genotyping		Phenotyping	
		ToxA	*ToxA-ToxB-*	Race 1	Race 4
2013	20	19	1	19	1
2014	83	4	79	4	79

Genotyping = characterization of isolates for the presence/absence of *Ptr ToxA* and *Ptr ToxB* genes (Andrie et al. 2007). Phenotyping = characterization of isolates by testing them on a tan spot differential set for their race structure (Lamari et al. 2003)

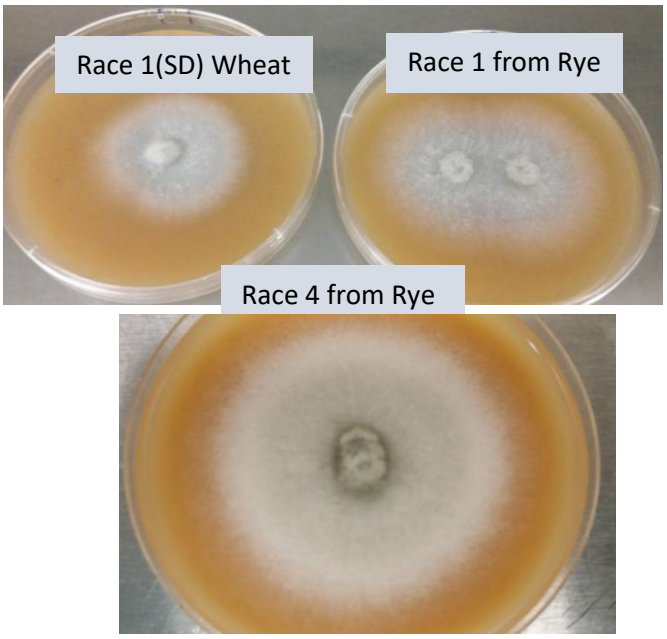

Figure 4.5. Mycelial growth of *P. tritici-repentis* isolates recovered from wheat and rye.

In our study, we analyzed 103 *Ptr* isolates from rye and identified race 1 and race 4 were prevalent on rye, which is not surprising because race 1 is the most prevalent race worldwide including the US Northern Great Plains (Ali and Francl, 2003; Benslimane et al., 2011; Lamari et al., 2003). It is possible that *Ptr* is surviving as an opportunistic pathogen/saprophyte on necrotic area caused by rye leaf spot pathogens, as most collected samples were also heavily infected with *Bipolaris sorokiniana*, *Stagnospora nodorum*, and *Alternaria spp* (data not shown). Earlier prevalence of *B. sorokiniana*, and *S. nodorum* on rye has also documented by American Pyhtopathological Society (1993). Previously we have reported race 1 on barley, another alternative host of *Ptr* and concluded its survival as a facultative saprophyte (Ali and Francl, 2001). Prevalence of race 4 on rye in the region validates our earlier results (Ali and Francl, 2003), observing race 4 population at a higher frequency on alternative hosts such as non-cereal grasses.

Conversely, the pathogen's isolates with high (race 1) and low aggressiveness (race 4) from alternative hosts such as Alti wild rye and smooth brome grass have also been documented in other studies (Krupinsky 1982, 1986, 1992; Howard and Morall, 1975; Hosford, 1971 and 1982). However, *Secale cereale* was not included in these studies.

In a first comprehensive study, we evaluated 211 rye genotypes representative of six continents against the tan spot pathogen's races 1 and 5 and their host selective toxins Ptr ToxA and Ptr ToxB. Our results suggest that rye genotypes exhibit diversity in reaction to tan spot caused by *Ptr* race 1 and race 5. However, susceptibility to race 1 and 5 in the evaluated genotypes is probably not due to *Ptr ToxA* and *Ptr ToxB* sensitivity as they lacked in *Tsn1* and *Tsc2* sensitivity genes needed for the two toxins. This suggests the role of unknown toxins or other factors in disease development. Further, the existence of race 1 and 4 on rye grown in South Dakota indicates it may have a minimal role in diversification of *Ptr* population and in the disease epidemiology in the region. However, planting race 5 susceptible rye germplasm in the region could change this scenario in the future. Therefore, it is imperative that tan spot resistant rye cultivars should be planted and periodic monitoring of the fungal population on rye crops would help in better management of tan spot throughout the region.

ACKNOWLEDGEMENTS

We like to thank Mr. Richard Gappert and Ms. Navjot Kaur for their help in greenhouse and lab work. The South Dakota Wheat Commission and USDA NIFA through SDSU Agricultural Experiment Station (AES) provided financial support for this research.

LITERATURE CITED

Aboudkhaddour, R., Kelly, T. T., and Stephen E. S. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. *Can J. Plant Pathol.* 35:256-268.

Abdullah, S., Byamukama, E., Langham, M., and Ali, S. 2016. First report of *Pyrenophora tritici-repentis* race 5 from South Dakota. *Plant Dis.* 100:2322.

Ali, S., and Francl, L. J. 2002. Race structure of *Pyrenophora tritici-repentis* isolates obtained from wheat in South America. *Plant Protection Science* (special issue) 38:302-304.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Dis.* 94:229-235.

Ali, S., Francl, L. J. and DeWolf, E. D. 1999. First report of *Pyrenophora tritici-repentis* race 5 from North America. *Plant Dis.* 83:591.

Ali, S., and Francl, L. J. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. *Plant Dis.* 85:580-584.

Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses. *Plant Dis.* 87:418-422.

Ali, S., and Langham, M. A. C. 2015. Reaction of five non-cereal grasses to five races and two host selective toxins of *Pyrenophora tritici-repentis. Plant Pathol. J.* 31:245-251.

American Phytopathological Society 1993. Diseases of Rye (*Secale cereale* L.). St. Paul, MN: APS.

Andrie, R. M., Pandelova, I. and Ciuffetti, L. M. 2007. A combination of phenotypic and genotypic characterization of *Pyrenophora tritici-repentis* race identification. *Phytopathology* 97:694-701.

Benslimane, H., Lamari, L., Abdelkader, B., Rachid, S., and Zouaoui, B. 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria. *Phytopathol. Mediterr* 50:203−211.

Burdon, J. J. 1993. The structure of pathogen populations in natural plant communities. *Annu. Rev. Phytopathol.* 31:305-323.

Bushuk, W. 2001. Rye production and uses worldwide. *J. Cereal Food World* 46:70-73.

Cox, C. M., Garrett, K. A., Cox, T. S., Bockus, W. W., and Peters, T. 2005. Reactions of perennial grain accessions to four major cereal pathogens of the Great Plains. *Plant Dis.* 89:1235-1240.

De Wolf, E. D., Effertz, R. J., Ali, S., and Francl, L. J. 1998. Vistas of tan spot research. *Can. J Plant Pathol.* 20:349–370.

Dinoor, A. 1974. Role of wild and cultivated plants in the epidemiology of plant diseases in Israel. *Annu. Rev. Phytopathol.* 12:413-436.

Effertz, R. J., Anderson, J. A., and Francl, L. J. 1996. QTLs associated with resistance to cholorosis induction by *Pyrenophora tritici-repentis* in adult wheat. *Can J. Plant Pathol.* 20:438-439.

Faris, J. D. and Friesen, T. L. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. *Theor. Appl. Genet* 111:386–392.

Faris D. J., Zhaohui, L. and Xu, S. S. 2013. Genetics of tan spot resistance in wheat. *Theor. Appl. Genet.* 126:2197-2217.

Hosford, R. M. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. *Phytopathology* 61:28-32.

Hosford, R. M. 1982. Tan spot - developing knowledge 1902-1981, virulent races and wheat differentials, methodology, rating systems, other leaf diseases, literature. In R. M. Hosford (Ed.), Tan Spot of Wheat and Related Disease Workshop (pp. 1-20). Fargo, ND: North Dakota Agric. Exp. Station.

Howard, R. J., and Morrall, R. A. A. 1975. The epidemiology of leaf spot disease in a native prairie. I. The progression of disease with time. *Can. J. Bot.* 53:1040-1050.

Krupinsky, J. M. 1982. Observation of the host range of isolates of *Pyrenophora trichostoma*. *Can. J. Plant Pathol.* 4:42-46.

Krupinsky, J. M. 1986. *Pyrenophora tritici-repentis*, *P. bromi*, and *Leptosphaeria nodorum* on *Bromus inermis* in the Northern Great Plains. *Plant Dis.* 70:61-64.

Krupinsky, J. M. 1992. Grass hosts of *Pyrenophora tritici repentis*. *Plant Dis.* 76:92–95.

Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. *Can. J. Plant Pathol.* 11:49-56

Lamari, L., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. *Phytopathology* 93:391-396.

Lamari, L., McCallum, B. D., and DePauw, R. M. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. *Phytopathology* 95:44-152.

Lepoint, P., Renard, M.-E., Legrève, A., Duveiller, E., and Maraite, H. 2010. Genetic diversity of the mating type and toxin production genes in *Pyrenophora tritici-repentis*. *Phytopathology* 100:474-483.

Moreno, M. V., Stenglein, S. A., Blatti, P. A., and Perollo, A. E. 2008. Pathogenic and molecular variability among isolates of *Pyrenophora tritici-repentis* causal agent of tan spot of wheat in Argentina. *European J of Plant Path.* 122:239-252.

Noriel, A. J., Xiaochum, S., Willimam, B., and Guihua, B. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. *Crop Science* 51:1059-1067.

Oliver, R. P., Cai, X., Wang, R. C., Xu, S. S., and Friesen, T. L. 2008a. Resistance to tan spot and Stagonospora nodorum blotch in wheat-alien species derivatives. *Plant Dis.* 92:150-157.

Oliver, R. P., Lord, M., Rybak, K., Faris, J. D., and Solomon, P. S. 2008b. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin sensitive cultivars. *Phytopathology* 98:488-491.

Postnikova E. N., and Khasanov B. A., 1998. Tan spot in Central Asia, pp.107–113. In: *Helminthosporium Blights of Wheat:* Spot Blotch and Tan Spot, El Batan, Mexico, edited by E. Duveiller, H. J. Dubin, J. Reeves, A. McNab. CIMMYT (International Maize and Wheat Improvement Center), Mexico, D.F., Mexico.

Rabinovich, S. V. 1998. Importance of wheat-rye translocations for breeding modern cultivars of *Triticum aestivum* L. *Euphytica* 100:323-340.

Saulescu, N. N., Ittu, M., Ciuca, M., Ittu, M., Serban, G., and Mustatea, P. 2011. Transferring useful rye genes to wheat using Triticales as a bridge. *Czech J. Genet. Plant Breed.* S 56-62.

Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press Co., New York. 538 pp.

Virdi S., Liu, Z. Overlander, M., Zhang, Z., Xu, S. S., Friesen, T. and Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. *G3: Genes, Genomes, Genetics;* DOI: 10.1534/g3.116.036525

Zhang, X., and Jin, Y. 1998. Sensitivity of Ptr ToxA and tan spot infection response in *Aegilops*/*Triticum* complex. *Can. J. Plant Pathol.* 20:415-418.

Zhang, X. Q., Wang, X. P., Ross, L., Hu, H., Gustafson, J. P., and Qualset, C. O. 2001. Rapid introduction of disease resistance from rye into common wheat by anther culture of a 6x triticale × nulli-tetrasomic wheat. *Plant Breeding* 120:39-42.

CHAPTER 5

TITLE: GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF *PYRENOPHORA TRITICI-REPENTIS* ISOLATES FOR THEIR RACE STRUCTURE AND REACTION OF WHEAT CULTIVARS TO TAN SPOT GROWN IN SOUTH DAKOTA

ABSTRACT

The fungus *Pyrenophora tritici-repentis* causes tan spot of wheat, an important leaf spotting pathogen in the US Northern Great Plains. Tan spot can cause significant yield loss up to 53% under suitable conditions for disease development in susceptible cultivars. The fungal isolates recovered from wheat and alternative hosts have been grouped into eight races based on their ability to produce two typical symptoms necrosis and chlorosis on tan spot wheat differential set. Further, the fungus produces three host selective toxins, Ptr ToxA, Ptr ToxB, and Ptr ToxC which are associated with necrosis and chlorosis symptoms. Races 1 through 5 have been reported from some states of the USA. However, information of *P. tritici-repetis* population race structure on wheat in South Dakota is lacking. A limited information on the reaction of commercially grown wheat cultivars to tan spot in the state is available. In this study, 569 single-spore *P. tritici-repentis* isolates recovered from wheat in South Dakota were evaluated for their race structure to utilize this information for developing reliable tan spot disease management strategies including resistant cultivars. Further we screened 45 wheat cultivars consisting of spring wheat (n= 32) and winter wheat (n=13) tan spot reaction using race 1 and race 5 to know if any cultivar was susceptible to both races and provide chances for their existence. All 569 isolates were characterized for *Ptr ToxA* and *Ptr ToxB* genes using the two toxins specific primers. Further, a subset of 134 of 569 isolates was characterized for their race identification on the tan spot wheat differential set. Out of 569 isolates, 510 isolates amplified *Ptr ToxA* gene. Only two of the isolates harbored *Ptr ToxB* gene. Based on the isolates molecular analysis, these 510 isolates could be

potentially of races 1, 2, 5, and 6. Rest of the 57 isolates did not amplify either of the two toxins genes and may be of race 3 and/or 4 depending on their phenotypic reaction. Based on phenotypic reaction of 134 isolates, 74.6% (n=100) were identified as race 1; 18.7% (25) as race 4; and 1.2% as race 5. Only one isolate was characterized as race 2. Six (4.5%) of the isolates did not fit under the current eight races as they produced necrosis on Glenlea and no chlorosis on 6B365, but they lacked in *Ptr ToxA* gene. The results of this study indicate diversity in the fungal populations and race 5 was observed for the first time in South Dakota. Presence of race 1 and race 5 warrants screening of wheat germplasm with both races for developing durable tan spot resistance cultivars. The isolates that did not fit under the 8 races are under investigation to determine if they produce any new toxin(s) responsible for necrosis symptoms.

Key words: Tan spot, *Triticum aestivum*, South Dakota state, race, virulence genes, Ptr ToxA and Ptr ToxB

INTRODUCTION

Wheat is the second most important crop in South Dakota, contributes significant revenue to the state economy, and provides employment opportunity to state population associated with wheat farming and associated industry. In 2016, wheat was planted on 2.7 million acres with the total production of 103.16 million bushels (NASS, 2016). Tan spot caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. is an important foliar disease of wheat worldwide and can impact wheat productivity ranging from 5-29% in South Dakota (Buchneau et al., 1983). The fungus has known to have a broad host-range in addition to its primary and economic host wheat and that including barley, oat, rye and many non-cereal grasses (Ali and Francl, 2003; Hosford, 1971; Krupinsky, 1992; Sprague, 1950). Tan spot symptoms on susceptible wheat cultivars include oval shaped tan necrotic lesions encircled with a chlorosis ring and pinhead size black spot in the center. In the US, Northern Great Plains, tan spot can cause significant yield loss up to 53% depending on the cultivar susceptibility level, inoculum level, plant growth stage at the time of infection and suitable environment for disease development (Hosford, 1982; Shabeer and Bockus, 1988). The yield losses are attributed due to low 1000 KWT, shriveled kernels, and number of grains/head results in yield loss (Shabeer and Bockus, 1988; Sharp et al., 1976). Currently, tan spot is generally managed through fungicide application and deployment of moderately susceptible cultivars due to lack of availability of resistant cultivars. However, excessive use of fungicide could increase fungicide insensitivity in the pathogen (Ciuffetti et al., 2014; FRAG UK 2016; Hahn and Leroch, 2015). The fungus survives from one growing season

to the next season primarily on previous year wheat crop residue (Hosford, 1982; Shabeer and Bockus, 1988). Tan spot on wheat was initially reported in the 1930's in North America and elsewhere (Conner, 1939; Mitra, 1934; Ito, 1930) but it was considered as a minor disease. In the early 1970's the disease has become major threat to wheat productivity, and the disease increase in the 1970's is anecdotally thought to be 1) change in tillage practices from conventional to zero or minimal that leave a large amount of residue hence contributing more inoculum for the coming growing season, 2) deployment of more tan spot susceptible cultivars with high yield potential, and 3) change in the pathogen virulence (DeWolf et al., 1998).

Virulence variation in *P. tritici-repentis* isolates have been observed based on their ability to produce necrosis and/or chlorosis symptoms in appropriate wheat differential genotype (Ali and Francl, 2003; Ali et al., 2010; Lamari and Bernier, 1989b; Lamari et al., 2003). Further, three host-selective toxins, Ptr ToxA, Ptr ToxB, and Ptr ToxC, were isolated from the fungus depending on pathogen race that are responsible for necrosis and chlorosis symptoms in toxin sensitive wheat genotypes (Abdullah et al., 2017 in press; Ciuffetti et al., 2010; Lamari et al., 2005). At present, eight races have been identified in the fungal populations recovered from wheat and alternative hosts (Ali and Francl, 2003; Lamari et al., 2003). Resistance to *P. tritici-repentis* in the host (wheat) is controlled by independent gene(s). Multiple races have been detected within the same area, race 1 is so far, the predominant race found in the studied areas (Aboukhaddour et al., 2013; Ali and Francl 2003; Ali et al., 2010; Bensalimane et al., 2011; Gamba et al., 2012; Lamari et al., 2003). Determination of the *P. tritici-repentis*

races prevalent in a specific region can be roughly estimated by evaluation of wheat cultivars grown commercially over a longer period with known races susceptibility. However, obtaining the complete inventory of the pathogen races is only possible through characterization of pathogen isolates using the available molecular and applied tools. Based on consensus of researchers involved in studying *P. tritici-repentis* race structure, a new race in the pathogen population can only be recognized/designated if the isolates produce necrosis and/or chlorosis symptoms should also produce a host-selective toxin associated with the symptoms by spore inoculation and it can be seen for eight currently identified races (Ciuffetti et al., 1998). At present, Ptr ToxA (associated with necrosis) and Ptr ToxB (chlorosis) genes specific molecular markers are available and can be utilized to determine if the isolates have *Ptr ToxA* and/or *Ptr ToxB* genes. Presence of *Ptr ToxA* and *Ptr ToxB* genes in the isolates provide us information that they may belong to races 1, 2, 5, 7 or absence of these genes indicate they may be of race 3 or 4 or a new race (Lamari et al., 2003). This limitation demands that the isolates further be tested on a wheat differential for the correct race identification.

It is important to obtain information on the virulence variation in the pathogen population for breeding durable disease resistance cultivars. Due to scarcity of complete tan spot resistant cultivars, the disease is mostly managed through growing moderately susceptible cultivars and fungicide application in the region. Though several effective fungicides against tan spot are available, the increase in cost of inputs, low wheat grain prices and risk of fungicides resistance in the pathogen population minimize the use of this management strategy. Deployment of tan spot resistance cultivars to reduce tan

spot impact on crop productivity is a reliable and environmental friendly approach; however, development of durable resistant cultivars requires sources of host resistance and knowledge of genetic variability in the pathogen population in the region. Very limited information is available on *P. tritici-repentis* race structure on wheat from South Dakota. Further, wheat cultivar (s) susceptibility to a particular *P*. *tritici-repentis* race can aid in its population increase and dominance in a particular region. The objectives of this study were 1) characterize *P. tritici-repentis* isolates recovered from wheat for their race structure in South Dakota and 2) screen wheat cultivars against tan spot grown in South Dakota.

MATERIAL AND METHODS

Leaves samples collection and recovery of *P. tritici-repentis* Isolates

Diseased wheat leaves exhibiting tan spot symptoms were randomly collected from 55 fields during 2012 (n=18), 2013 (n= 19), and 2014 (n=18) growing seasons. Fifteen to twenty leaves were collected from each field. The samples were comprised of hard red winter and hard red spring wheat. The sampling locations included both SDSU experimental research stations and commercial wheat fields. The samples were collected when the crop was at milk-stage in most of the sampled fields. All leaf samples were cut into about 2 cm long segments with at least one leaf spot, placed in paper bags (one sample/bag) and stored in the refrigerator until the isolations were made. The recovery of the fungus *P. tritici-repentis* isolates from the leaves samples were done using the method of Ali and Francl (2001). Briefly, 40-50 leaf segments were plated (8-10 segments/plate) on three layers of moist filter paper Whatman #1. The plates were incubated under fluorescent light for 24 hours at room temperature (22+-1 ^{o}C) and 24 hours dark at 16 ^{o}C to initiate conidiophores and conidia formation, respectively. The incubated leaf segments were examined under stereoscope for the fungal conidia and then 10 -15 single spores were picked-off using a flamed steel needle and transferred individually onto V8-PDA (150 ml of V8 juice, 10 g of Difco PDA, 10 g of Difco agar, 3 g of calcium carbonate, and 850 ml of distilled water) plates (Lamari and Bernier, 1989a). The cultures were grown for 5-6 days in the dark and then stored at -20^{o}C using the procedure of Jodhal and Francl (1992) until they were characterized for their race structure. In total 569 isolates were recovered over 2012 (n=138), 2013 (n=176) and

2014 (n=255) three years' period. Eight *P. tritici-repentis* isolates recovered from wheat leaves samples collected in Nebraska were also included in the study.

Genotypic characterization of *P. tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes

DNA extraction and PCR assays

Fresh cultures of all 569 *P. tritici-repentis* isolates were obtained by growing them individually on V8-PDA medium, using their frozen dry plugs stored at -20^{o}C, for 5 days. The mycelia of each isolate were removed from the agar surface with a sterile scalpel and placed in a 2-ml microfuge tube, dried overnight in a water bath at 37°C and then ground into a fine powder using a first prep machine (Retsch MM 301). DNA from all the isolates was recovered by following the protocol of Moreno et al., (2008). The DNA concentration was normalized to 25ng/µl using a Nano drop machine (NanoDrop 1000 UV/Vis Spectrophotometer, Counterpane Inc. Tacoma, WA, USA) and run in a 0.8% agarose gel to verify the DNA quality. Genotype of the *P. tritici-repentis* isolates for *Ptr ToxA and Ptr ToxB* genes were determined by using the *Ptr ToxA* and *Ptr ToxB* genes specific primers developed by Andrie et al., (2007) (Appendix table 1a-c). The conformity of the isolates of *P. tritici-repentis* was verified by using two *P. tritici-repentis* mating type genes specific primers (Lepoint et al., 2010). PCRs for the toxins genes and isolates conformity specific markers were performed in 20 µl volume; 2 µl genomic DNA (25ng/ µl), 0.8 µl of each primer (10mM), 0.5 µl dNTP (200 µM), 2 µl 10 x thermophol buffer, 0.2 µl 10 U/ml *taq* polymerase and 13.7 µl water. PCR reaction was conducted in a S-1000 thermal cycler (BioRad, USA) using amplification steps of 94°C for 1 minute,

followed by 30 cycles of 94°C for 45 seconds, 55°C for 30 seconds and 72°C for 1 minute with final extension of 72°C for 7 minutes. The amplified products were electrophoresed on 1.5% agarose gels and scored with reference to 1 Kb ladder (New England Biolabs, USA). Two known *P. tritici-repentis* isolates, one each of race 1 (Pti2) and race 5 (DW7) were included as positive checks for *Ptr ToxA* and *Ptr ToxB* genes, respectively.

Phenotypic characterization of 134 *P. tritici-repentis* isolates

Tan spot wheat differentials seedlings and standard fungal isolates

The seedlings of four tan spot wheat differential genotypes Salamouni, Glenlea, 3B365, and 6B662 (Lamari et al., 2003) were raised in 3 x 9 cm plastic containers (Stuewe & Sons, Inc. 31933 Rolland Drive, Tangent, OR 97389 USA) filled with Sunshine Mix 1 (770 Silver Street Agawam, MA, USA). Three seedlings per container were maintained throughout the experimentation. The plants were watered and fertilized as needed. The plants were kept on a greenhouse bench at 22^{o}C for 16 hours' photoperiod throughout the experimentation. The fungal isolates Pti2 (race 1) and DW7 (race 5) were included as positive checks for validation of inoculation procedure and race identification.

Inoculum production, inoculation and isolates race characterization

The inoculum preparation and inoculations were performed as described in Ali and Francl (2001). Briefly, fresh cultures of a subset of randomly selected 134 of the 569 isolates genotyped for *Ptr ToxA* and *Ptr ToxB* genes were obtained by plating their frozen dry plugs on fresh V8-PDA medium plates. The plates were incubated in dark for 5 days or until the colony growth diameter reached about 5 cm. The plates were then

flooded with distilled sterile water and the mycelial growth was flattened with the bottom of a sterile test tube. The excess water was poured off from the plates and exposed them for 24 hours to fluorescent light at room temperature (~22^{o} C) and then for 24 hours in dark at 16^{o}C for spore production. The conidia were harvested with a plastic looped needle by adding about 30 ml of distilled sterile water in each plate and the final spore concentration was adjusted to 2500 spores/ml prior to inoculations. Two weeks old seedlings of all 4 differentials genotypes Glenlea, 6B365, 6B662, and Salamouni with three replications were inoculated individually with all 134 isolates by spraying their spore suspension with a handheld CO_2 pressurized sprayer (Preval, Chicago Aerosol, 1300 E. North Street, Coal City, IL 60416, USA). The seedlings were placed in a humidity chamber at 100% humidity for 24 hours for enhancing the chances of fungal infection. Thereafter, the seedlings were moved to a greenhouse bench for seven days for symptom development. The isolates were grouped under appropriate race based on their ability to produce necrosis and chlorosis symptoms on the differentials genotype (Lamari et al., 2003) (Appendix table 5.1a-c).

Evaluation of wheat cultivars for their reaction to tan spot race 1 and 5 and Ptr ToxA grown in South Dakota

Seedlings of forty-one wheat cultivars that are/or have been planted as a popular variety at one point in South Dakota were inoculated with *Ptr* race 1 and 5. The cultivars seed was kindly provided by SDSU spring wheat and winter wheat breeding programs. The goal of evaluation of these cultivars was to see if any of the cultivars are susceptible to both races 1 and 5 or cultivars play any role in *P. tritici-repentis* race 5

prevalence, recently observed in South Dakota (Abdullah et al., 2016). Additionally, we evaluated 4 advanced wheat lines of hard red spring wheat in this study. Tan spot wheat differential genotypes Glenlea, 6B365, 6B662, and Salamouni were included in the experiment as checks for verification of successful inoculation procedure and disease rating. The seedlings of all 45 wheat genotypes were inoculated individually by using race 1 and race 5 spore suspensions as described above. Nine seedlings (3 seedlings/container) of each cultivar were inoculated and the experiment was repeated once. The inoculated seedlings were rated for their reaction based on 1-5 rating scale (Lamari and Bernier 1989a).

All Forty-five wheat genotypes including 4 SDSU breeding lines (Total 45) were evaluated against Ptr ToxA as described in Faris et al. (1996) (Table 5.3a-b). Briefly, fully expanded second leaf of two weeks old seedlings of all 45 genotypes were infiltrated with Ptr ToxA (10ug/ml), using a needleless syringe and infiltrated leaf area was marked by a sharpie non-toxic permanent marker. Three leaves of each genotype were infiltrated with the toxin. The seedlings were rated as sensitive/insensitive based upon the presence/absence of necrosis, respectively. Wheat differential lines Glenlea (sensitive to Ptr ToxA) and Salamouni (insensitive to Ptr ToxA) were included in the experiment. Ptr ToxA was kindly provided by Dr. Steven. Mienhardt, Department of Plant Pathology, North Dakota State University, Fargo, ND.

RESULTS AND DISCUSSION

Molecular evaluation of *Pyrenophora tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes

All 569 isolates produced DNA bands to *P. tritici-repentis* mating types specific primers hence re-confirmed the identity of the isolates. Further, DNA bands (585bp and 295bp) specific to *Ptr ToxA* and *Ptr ToxB* genes were amplified from isolates Pti2 (race 1) and DW7 (race 5), respectively, that validating the success of PCR assay (Figure 5.1). Based on the results of genotypic analysis, the isolates were divided into three groups that included 1) isolates with *Ptr ToxA* gene; 2) isolates with *Ptr ToxB* gene; and 3) the isolates without both *Ptr ToxA* and *Ptr ToxB* genes (Table 5.1). The isolates (n = 510, 89.5%) belong to group 1 potentially be of race 1, 2, and 8, as these races carry *Ptr ToxA* gene, but lack in *Ptr ToxB* gene in these isolates eliminated the possibility of race 8 (Lamari et al., 2003). Further, the frequency of isolates with *Ptr ToxA* gene were higher in all three years, 2012 (n = 119, 86.2%), 2013 (n = 165 (93.8%), and 2014 (n = 226, 88.3%) (Table 5.1). All eight isolates from Nebraska amplified *Ptr ToxA gene*. The isolates with *Ptr ToxB* gene was only observed in 2013. Presence of *Ptr ToxA* gene at high frequency in the evaluated isolates provides an indication of predominance of race 1 in South Dakota as it has been observed in various independent studies conducted in different countries (Ali and Francl, 2003; Benslamane et al., 2011; Gamba et al., 2012; Sarova et al., 2005), however, exact nature of the isolates race information is only possible through their phenotyping on the differential set (Lamari et al., 2003). Only two (0.4%) isolates that exhibited *Ptr ToxB* gene could possibly be of either race 5 or race 6 depending on the phenotypic reaction of these isolates if they carry *Ptr ToxC* gene on

wheat differential genotypes. Though only two isolates with *Ptr ToxB* gene from one location in 2013 were observed, they signify the prevalence of either race 5 or race 6, previously not observed in South Dakota (Abdullah et al., 2016). Lack of molecular markers for *Ptr ToxC* gene that separate race 1 from 2; race 3 from race 4; and race 5 from 6 is a bottleneck in grouping the isolates into absolute race based on PCR results. The isolates belong to group 3 that lack in *Ptr ToxA* and *Ptr ToxB* genes may belong to race 3 and 4, or they may be representative of a new race as it has been reported in previous studies (Abdullah et al.,2017; Ali et al., 2010; Andrie et al., 2007; Mironenko et al., 2015). Moreover, the isolates without *Ptr ToxA and PtrToxB* genes were observed in all three years 2012 (19 isolates, 13.8%), 2013 (9 isolates, 5.1%) and in 2014 (29 isolates, 11.7%) but they varied in their proportion (Table 5.1). Our results indicate, majority (n=510) of the isolates collected over the three years' period from South Dakota carry *Ptr ToxA* gene and followed a similar pattern that have been reported in previous studies (Abdullah et al., 2017 in press; Aboukhaddour et al., 2013; Friesen et al., 2005). The whole genome of *P. tritici-repentis* has been sequenced (Birren et al., 2007; Manning et al., 2013) and it may help in the development of molecular markers for race 3 (*Ptr ToxC*) and 4 in near future and reduces the dependency of phenotyping, a time-consuming procedure and environment sensitive and requires a skilled person to observe phenotypic reaction.

Table 5.1. Genotypic analysis of 569 *P. tritici-repentis* isolates for *Ptr ToxA* and *Ptr ToxB* genes prevalent on wheat in South Dakota

Year	Isolates tested	With *Ptr ToxA* gene	With *Ptr ToxB* gene	Without *ToxA* and *ToxB* genes
2012	138	119 (86.2%)	0 (0%)	19 (13.8%)
2013	176	165 (93.8%)	2 (1.1%)	9 (5.1%)
2014	255	226 (88.3%)	0 (0%)	29 (11.7%)
Total	**569**	**510 (89.5%)**	**2 (0.4%)**	**57 (10.2%)**

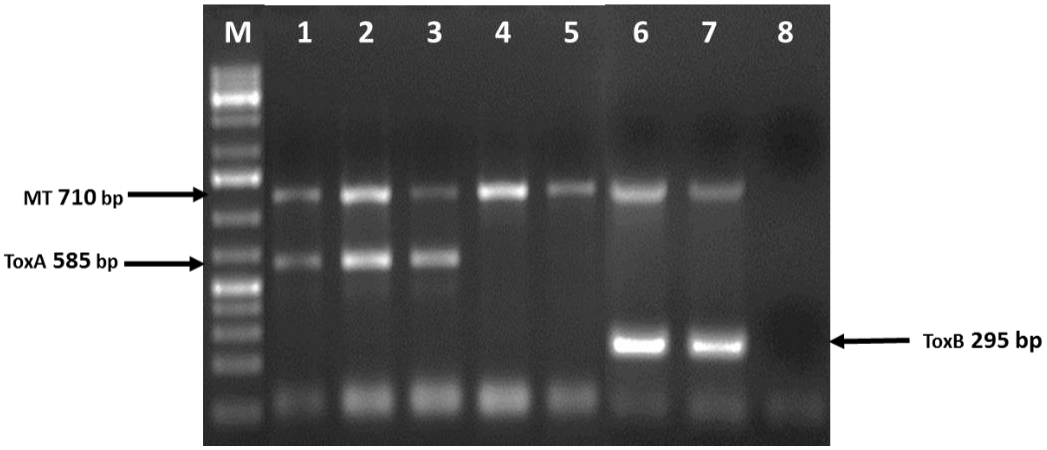

Figure 5.1. Gel picture of the *P. tritici-repentis* isolates showing the presence of *ToxA*, *ToxB* and *Mating type* (*MT*) genes of *Pyrenophora tritici-repentis*. Lane 1-3: *Ptr ToxA* gene from isolates SD13-101, SD12-S26-SN27-P2 from this study (unknown), and Pti2 (race1^{ve+}control); Lane 4-5: isolates 12-S11-SN11-P7.2 and 12-S11-SN11-P7.3 without *ToxA/ToxB*; Lane 6-7: *PtrToxB* from isolate of race 5 (SD13-103) and DW-7 (race 5^{ve+}control) and Lane 8: water as negative control.

Phenotypic evaluation of *P. tritici-repentis isolates* for their race identification

A subset of randomly chosen 134 of 569 *P. tritici-repentis* isolates studied for *Ptr ToxA* and *Ptr ToxB* genes were further characterized for their race structure on tan spot wheat differential set. As expected, race 1 isolate “Pti 2” produced necrosis and chlorosis and race 5 isolate “DW7” produced chlorosis on appropriate differential

genotypes hence confirm the validity of the isolates and inoculation procedure. In this study, the evaluated 134 isolates were grouped into races 1, 2, 4, and 5 (Table 5.2) indicate prevalence of diverse population of *P. tritici-repentis* in on wheat in South Dakota. Among these 4 races, majority of the isolates from 2012 (40%), 2013 (84%), and 2014 (80%) were identified as race 1. However, their proportion was varied from year to year (Table 5.2). Prevalence of race 1 at high frequency in the state is not surprising as most of the cultivars grown or had been grown in the state exhibit some level of susceptibility to tan spot race 1 (Table 5.3 a-b). Our results confirm the previous studies that observed race 1 was the most prevalent race worldwide (Ali and Francl, 2003; Ali et al., 2010; Lamari et al., 2003; Engle et al., 2006; Gamba et al., 2012; Sarova et al., 2005). It is possible that the dominance of race 1 in various countries may be due to deployment of tan spot susceptible cultivars to race 1 on a larger area, as it is documented from various independent studies regarding germplasm screening to multiple races and majority of genotypes were susceptible race 1 as compared to race 3 and/or 5 (Ali et al., 2008; Singh et al., 2006). However, this situation does not eliminate the possibility of other races prevalence at low frequency.

Twenty-five (18.65%) of the isolates were identified as race 4 and were ranked second in their prevalence in all three years, 2012 (32%), 2013 (13%), and 2014 (19%). Prevalence of race 4 on wheat ranging from 13-32% depending on the isolates collection year is a bit higher as compared to previous studies where about 5% of the isolates were race 4 (Ali and Francl, 2003; Benslimane et al., 2011; Lamari and Bernier 1989b; Sarova et al., 2005). However, race 4 with high frequency has been reported on the fungal

alternative host's non-cereal grasses in the US northern Great Plains (Ali and Francl, 2003). Recently, we characterized the fungal isolates recovered from rye, an alternative host of *P. tritici-repentis* and seventy-eight percent of 103 isolates were of race 4 (See chapter 4). Recovery of race 4 isolates a bit higher in frequency on wheat in this study may be coming from rye and surviving as a weak pathogen on wheat, as rye is generally planted overlaps wheat plots in the state.

Only 3% of the isolates evaluated were identified as race 5 in this study (Table 5.2). Race 5 was detected only in 2013 with very low frequency but it was reported for the first time from South Dakota (Abdullah et al., 2016). Prior to its discovery in South Dakota, it was reported only from North Dakota in the USA (Ali et al., 1999). Only one isolate was identified as race 2 of 134 isolates characterized. Surprisingly 6 of 31 isolates that lacked in Ptr *ToxA* and *Ptr ToxB* genes and seemingly are of either race 3 or race 4 based on genotypic data did not fit under any of the currently identified 8 races as they produced necrosis (Figure. 5.2) like race 2 on Ptr ToxA sensitive differential genotype Glenlea and avirulent reaction on the rest three differential genotypes. Recovery of these type of isolates are not surprising to us as they have been reported in many other independent studies (Abdullah et al., 2017; Ali et al., 2010; Andrie et al., 2007; Postnikova and Khasanov, 1998). Occurrence of necrosis producing isolates without *Ptr ToxA* gene on the Ptr ToxA sensitive wheat genotype indicate that they may contain different toxin(s) from Ptr ToxA responsible for necrosis symptoms. These isolates further need to be investigated for their mechanism of host-pathogen interaction.

Table 5.2. Race characterization of *P. tritici-repentis* isolates prevalent on wheat in South Dakota

Year	Isolates tested	Race 1	Race 2	Race 4	Race 5	Nec+
2012	25	10 (40%)	1 (4%)	8 (32%)	0 (0%)	6 (24%)
2013	62	52 (84%)	0 (0%)	8 (13%)	2 (3%)	0 (0%)
2014	47	38 (80%)	0 (0%)	9 (19%)	0 (0%)	0 (0%)
Total	**134**	**100 (74.62%)**	**1 (0.74%)**	**25 (18.65%)**	**2 (1.49%)**	**6 (4.4%)**

Nec+ = *P. tritici-repentis* isolates without *Ptr ToxA* and *Ptr ToxB* gene but produce necrosis on Ptr ToxA sensitive genotype Glenlea.

Figure 5.2. Isolate SD-12-S11-SN11-P7.2 (Lacks in *Ptr ToxA* gene induced necrosis on Glenlea (A) but no chlorosis on 6B365(B)

Reaction of wheat cultivars to tan spot (*P. tritici-repentis* race 1 and race 5, and Ptr ToxA)

In this study, we evaluated total 45 wheat cultivars (hard red spring = 32 and winter hard red = 13) including four advanced lines to tan spot using race 1 and race 5 to determine potentially prevalent races in the region due to their susceptibility. Wheat cultivars belong to both spring wheat and winter wheat classes exhibited diverse reaction, susceptible to resistant, to both races (Table 5.3 a and b). In general, higher number of spring wheat cultivars than winter wheat cultivars exhibited susceptibility to race 1 and race 5. Six of 32 spring wheat cultivars/lines, Forefront, LCS Breakaway, Norden, Russ, Select, and SD4189 exhibited susceptibility to both races 1 and 5 (Table 5.3a). Fourteen cultivars showed susceptible to moderately susceptible reaction to race

5 only as compared to 10 cultivars were susceptible to only race 1. The spring wheat cultivars susceptible to both races 1 and 5 could play a significant role in the development of new virulent race(s) such as races 6 (Ptr ToxB and Ptr ToxC), 7 (Ptr ToxA and Ptr ToxB), and 8 (Ptr ToxA, Ptr ToxB, and Ptr ToxC) as they are the combination of race 1 (Ptr ToxA and Ptr ToxC) and race 5 (Ptr ToxB). The fungus *P. tritici-repentis* is a residue borne in nature and present almost all the time on wheat residue in South Dakota due to minimum tillage practices and this situation provides ample opportunity to the fungus for sexual reproduction. If the two races (1 and 5) are lying in the same leaf and/or in different plants within the same field, then there is a great chance of recombination among the two races through sexual production and a vast risk of evolving a new race. This might make tan spot management more difficult.

In contrast to spring wheat, about 50% (n=7) winter wheat cultivars exhibited susceptibility to tan spot race 1 and developed lesion type 3-3.8 (moderately susceptible) except Ideal (lesion type 4; susceptible). All genotypes developed lesion type 1-2 (moderately resistant to resistant) to race 5 (Table 5.3b). The results indicate that spring wheat cultivars susceptible to both races may be the potential sources for their survival in the region. Though race 5 was detected from one location in 2013 but monitoring the fungal isolates on wheat cultivars susceptible to both races or race 5 may provide better information on race 5 population prevalent in the state. Resistant reaction of majority winter wheat cultivars to race 5 minimze their role in race 5 establishment.

Table 5.3a. Reaction of hard red spring wheat cultivars to *P. tritici-repentis* race 1, race 5, and Ptr ToxA (Tan spot).

Number	Cultivar/Line	*P. tritici-repentis* races				Ptr ToxA
		Race 1		Race 5		
		Lesion type	Reaction	Lesion type	Reaction	
1	Advance	1.8	MR	2.3	MR	+
2	Barlow	1.5	R	1.9	MR	-
3	Breaker	1.0	R	2.0	MR	+
4	Brick	1.0	R	2.2	MR	+
5	Briggs	2.0	MR	2.6	MR	+
6	Chris	1.0	R	4.3	S	-
7	Elgin-ND	1.1	R	3.8	MS	+
8	Faller	1.0	R	3.6	MS	-
9	Forefront	3.7	MS	4.4	S	+
10	LCS Albany	1.1	R	1.4	R	+
11	LCS Breakaway	4.0	S	4.0	S	-
12	LCS Powerplay	1.2	R	1.8	MR	+
13	Linkert	2.0	MR	4.1	S	-
14	Mott	3.5	MS	1.8	MR	-
15	Norden	3.7	MS	4.0	S	+
16	Oxen	3.1	MS	1.1	R	+
17	Prosper	2.0	MR	3.6	MS	-
18	RB07	1.7	MR	1.3	R	+
19	Rollag	1.1	R	3.9	S	-
20	Russ	3.9	S	4.0	S	+
21	Sabin	1.3	R	1.0	R	+
22	Samson	1.1	R	1.2	R	-
23	SD4189	3.8	MS	4.0	S	+
24	SD4215	1.9	MR	1.0	R	+
25	SD4148	1.6	MR	3.0	MS	+
26	SD4011	1.1	R	2.1	MR	+
27	Select	4.0	S	3.8	MS	+
28	SY Soren	1.0	R	1.0	R	-
29	Transverse	3.3	MS	2.1	MR	+
30	Vantage	1.7	MR	1.3	R	-
31	Velva	1.2	R	3.8	MS	-
32	WB Mayville	4.0	S	1.1	R	+

Table 5.3b. Reaction of hard red winter wheat cultivars to *P. tritici-repentis* race 1, race 5, and Ptr ToxA (Tan spot).

Number	Cultivar/Line	*P. tritici-repentis* races				Ptr ToxA
		Race 1		Race 5		
		Lesion type	Reaction	Lesion type	Reaction	
1	Alice	3.0	MS	1.1	R	+
2	Expedition	3.3	MS	1.7	MR	+
3	Freeman	1.6	MR	1.0	R	-
4	Grainfield	3.0	MS	1.0	R	+
5	Ideal	4.0	S	1.0	R	+
6	Lyman	1.9	MR	1.0	R	+
7	Millennium	2.8	MR	1.2	R	+
8	Overland	3.2	MS	1.2	R	+
9	Redfield	2.1	MR	1.0	R	-
10	SY Rowyn	3.0	MS	1.2	R	+
11	Sy Wolf	1.0	R	1.0	R	-
12	WB Matlock	3.8	MS	1.3	R	+
13	Wesley	2.2	MR	1.0	R	-

Wheat differentials Glenlea developed necrosis and Salamouni showed insensitivity to Ptr ToxA as expected and verified the toxin sensitivity and the success of the toxin infiltration procedure. All 45 wheat cultivars varied in their reaction to Ptr ToxA as they developed either necrosis (sensitive) or no necrosis (insensitive). We did not find any correlation between the susceptibility-sensitivity and resistance-insensitivity to the toxin (Table 5.3a and b). Eight spring wheat cultivars were susceptible to fungus (race 1) and sensitive to the toxin (Ptr ToxA) and two cultivars were susceptible but insensitive to the toxin. Also, 10 cultivars were resistant to race 1 and insensitive to the toxin; whereas 12 cultivars were resistant to race 1 but sensitive to Ptr ToxA (Table 5.3a). For example, spring wheat cultivar LCS Breakway showed susceptibility (lesion type 4) to race 1 (Ptr ToxA producers) but exhibited insensitivity to

the toxin; where cultivar Select developed susceptible reaction (lesion type 4) to race 1 and also exhibited necrosis to the toxin. Seven winter wheat cultivars developed susceptible reaction to race 1 were also developed necrosis (sensitive) to Ptr ToxA. Four cultivars showed resistance to race 1 and insensitivity to the toxin; where 2 winter wheat cultivars exhibited resistant reaction to race 1 but were sensitive to Ptr ToxA. Our results confirm previous reports where wheat genotypes susceptible to tan spot and Ptr ToxA sensitive; susceptible and insensitive; resistant and sensitive; and resistant and insensitive were observed (Abdullah et al., 2017; Ali et al., 2010; Noriel et al., 2011; Oliver et al., 2008).

The results of this study indicate that a diverse population of *P. tritici-repentis* exists in wheat in South Dakota. Race 5 was observed for the first time in South Dakota. Spring wheat cultivars susceptible to race 1 and 5 could play a role in the development of more virulent races if race 1 and race 5 are present on the same leaf or plant and go through the sexual recombination. Additionally, isolates lack in Ptr ToxA need to be further investigated for their mechanism of pathogenicity/virulence to be sure that the cultivars resistant to race 1 and 5 also provide protection against these isolates. It is imperative to monitor the pathogen population periodically in the region. Also, wheat breeding material should be screened against both races prior to using in the development of tan spot resistant cultivars.

ACKNOWLEDGEMENTS

We like to thank Mr. Richard Gappert and Ms. Navjot Kaur for their help in field, greenhouse, and lab work. The South Dakota Wheat Commission and USDA NIFA through SDSU Agricultural Experiment Station (AES) provided financial support for this research.

LITERATURE CITED

Abdullah, S., Byamukama, E., Langham, M. G. H., and Ali, S. 2016. First report of *Pyrenophora tritici-repentis* race 5 from South Dakota. *Plant Dis.* 100:2322.

Abdullah, S., Byamukama, E., Langham, M. G. H., S. K. Sehgal and Ali, S.2017. Characterization of *Pyrenophora tritici-repentis* (tan spot of wheat) races in Baltic States and Romania (PPJ-OA-10-2016-0214). *Plant Pathol. J.* 33: 33-139.

Aboukhaddour, R., Turkington, T. K., and Strelkov, S.E. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada, *Can. J. of Plant Pathol.* 35:256-268.

Ali, S., Francl, L. J. and De Wolf, E. D 1999. First Report of *Pyrenophora tritici-repentis* race 5 from North America. *Plant Dis.* 83:591.

Ali, S., and Francl, L. J. 2001. Recovery of *Pyrenophora tritici-repentis* from barley and reaction of 12 cultivars to five races and two host-specific toxins. *Plant Dis.* 85:580-584.

Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses. *Plant Dis.* 87:418-422.

Ali, S., Singh, P. K., McMullen, Mergoum, M. P. M., and Adhikari T. B. 2008. Identification of New Sources of Resistance to Multiple Leaf Spotting Diseases in Wheat Germplasm. *Euphytica* 159:167-179.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. *Plant Dis.* 94:229–235.

Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007. A combination of phenotypic and genotypic characterization *Pyrenophora tritici-repentis* race identification. *Phytopathology* 97:694-701.

Birren, B., Lander, E., Galagan, J., Nusbaum C., Devon, K., Ma, L.-J., Jaffe, D., Butler, J., Alvarez, P., Gnerre, S., Grabherr, M., Kleber, M., Mauceli, E., Brockman, W., MacCallum, I.A., Young, S., LaButti, K., DeCaprio, D., Crawford, M., Koehrsen, M., Engels, R., Montgomery, P., Pearson, M., Howarth, C., Larson, L., White, J., Yandava, C., Kodira, C., Zeng, Q., O'Leary, S., Alvarado, L., Ciuffetti, L., Pandelova, I. 2007. *Pyrenophora* genome project. Broad Institute of Harvard and MIT.

Benslimane, H., Lamari, L., Benbelkacem, A., Sayoud, R., and Bouznad, Z. 2011. Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identification of a new virulence type. *Phytopathol.* Mediterr. 50:203−211.

Buchenau, G. W., Smolik, J. D., Ferguson, M. W., and Cholick, F. A. 1983. Wheat diseases in South Dakota. Ann. Wheat Newsletter 29: 147.

Ciuffetti, L. M., Francl, L. J., Ballance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., and Rasmussen, J. B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction, *Can. J. of Plant Pathol.* 20:421-424.

Ciuffetti, L. M., Manning, V. A., Pandelova, Betts, I., M. F., and Martinez, J. P. 2010. Host-selective toxins, Ptr ToxA, and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*- wheat interaction. *New Phytologist* 187:911-919.

Ciuffetti, et al., 2014. *Pyrenophora tritici-repentis*: A plant pathogenic fungus with global impact. n book: Genomics of Plant-Associated Fungi: Monocot Pathogens, Chapter: *Pyrenophora tritici-repentis*: A Plant Pathogenic Fungus with Global Impact, Publisher:

Springer-Verlag Berlin Heidelberg, Editors: Ralph Dean, Ann Lichens-Park, Chittaranjan Kole, pp.1-39.

Conners, I. L. 1939. Nineteenth annual report of the Canadian Plant Disease Survey 19:12-14.

De Wolf, E. D., Effertz, R. J., Ali, S., Francl, L. J. 1998. Vistas of tan spot research. *Can. J Plant Pathol.* 20:349–370.

Engle, J. S., Madden, L. V., and Lipps, P. E. 2006. Distribution and pathogenic characterization of *Pyrenophora tritici-repentis* and *Stagonospora nodorum* in Ohio. *Phytopathology* 96:1355-1362.

Faris, J. D, Anderson, J., Francl, L. J, Jordahl, J. G.1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology.* 86: 459–463. doi: 10.1094/Phyto-86-459.

FRAG, 2016. Fungicide resistance management in cereals. p 13.

Friesen T. L., Ali S., Klein K. K., Rasmussen J. B., 2005. Population genetic analysis of a global collection of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. *Phytopathology* 95:1144-1150.

Gamba, F. M., Strelkov, S. E., and Lamari L. 2012. Virulence of *Pyrenophora tritici-repentis* in the southern cone region of south America. *Can. J. of Plant Pathol.* 34:545–550.

Hahn, M., and Leroch, M. 2015. Multidrug Efflux Transporters. Fungicide resistance in plant pathogens. pp 233-248.

Hosford, R. M. 1971. A form of *Pyrenophora trichstoma* patho¬genic to wheat and other grasses. *Phytopathology* 61:28-32.

Hosford, R. M. Jr. 1982. Tan spot - Developing knowledge 1902 - 1981. In Tan Spot of Wheat and Related Diseases Workshop. July. 1981. Fargo, N.D. Edited by Hosford, R.M.Jr. North Dakota Agricultural Experimental Station, North Dakota State University, Fargo, N.D. pp:1-24.

Ito, 1930. On some new ascigerous stages on the species *Helminthosporium* parasitic on cereals. Proceedings of the Imperial Academy of Tokyo. 6:352-355.

Jordahl, J. G., and Francl, L. J. 1992. Increase and storage of cultures of *Pyrenophora triticirepentis.* Page 109 in: Advances in Tan Spot Research. L. J. Francl, J. M. Krupinsky, and M. P. McMullen, eds. North Dakota Agric. Exp. Stn., Fargo.

Krupinsky, J. M. 1992. Grass hosts of *Pyrenophora tritici repentis*. *Plant Dis.* 76: 92–95.

Lamari, L., and Bernier, C. C. 1989a. Evaluation of wheat for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. *Can. J. Plant Pathol.* 11:49-56.

Lamari, L. and Bernier, C. C. 1989b. Virulence of isolates of *Pyrenophora tritici-repentis* on eleven wheat cultivars and cytology of the differential host reaction. *Can J. Plant Path.* 11:284-290.

Lamari, L., Srelkove, S. E., Yahyaoui. A., Orabi, J., and Smith, R. B. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one to one relationship in tan spot of wheat. *Phytopathology* 93:391-396.

Lamari L., Strelkov S. E., Yahyaoui A., Amedov M., Saidov M., et al. 2005. Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road. *Can. J. Plant Pathol.* 27:383–388.

Lepoint, P., Renard, M. E., Legrève, A., Duveiller, E. and Maraite, H. 2010. Genetic diversity of the mating type and toxin production genes in *Pyrenophora tritici-repentis*. *Phytopathology* 100:474-483.

Manning, V. A., Pandelova, I., Dhillon, B., Wilhelm, L. J., Goodwin, S. B., Berlin, A. M., Figueroa, M., Freitag, M., Hane, J. K., Henrissat, B., Holman, W. H., Kodira, C.D., Martin, J., Oliver, R. P., Robbertse, B., Schackwitz, W., Schwartz, D. C., Spatafora, J. W., Turgeon, B. G., Yandava, C., Young, S., Zhou, S., Zeng, Q., Grigoriev, I. V., Ma, L. J., Ciuffetti, L. M., 2013. Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence *G3* (Bethesda), 41–63.

Mironenko, N. V., Baranova, O. A., Kovalenko, N. M. and Mikhalova, L. A. 2015. Frequency of ToxA gene in North Caucasian and North-West Russian populations of *Pyrenophora tritici-repentis*. *Mikol. Fitopatol.* 49:325–329.

Mitra, M. 1934. A leaf spot disease of wheat caused by *Helminthosporium tritici-repentis* Died. *Ind. J. Agric. Sci*. 4:692-700.

Moreno, M. V, Stenglein, S. A., Blatti, P. A., and Perollo, A. E. 2008. Pathogenic and molecular variability among isolates of *Pyrenophora tritici-repentis* causal agent of tan spot of wheat in Arjentina. *Euro. J. of Plant Path.* 122:239-252.

National Agricultural Statistics Service (NASS) 2016. Crop Production (August, 2016), Agricultural Statistics Board, United States Department of Agriculture (USDA).

Noriel, A. J., Xiaochum, S. Willimam, B., and Guihua, B. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. *Crop Science.* 51:1059-1067.

Oliver, R. P., Lord, M., Rybak, K., Faris, J. D., and Solomon, P. S. 2008. Emergence of tan spot disease caused by toxigenic *Pyrenophora tritici-repentis* in Australia is not associated with increased deployment of toxin sensitive cultivars. *Phytopathology* 98:488-491.

Postnifova, E. N., and Khasanov, B. A. 1998. Tan spot in Central Asia. Pages 107-113 In: Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. E. Duveiller, H. J. Dubin, J. Reeves, and A. McNab, eds. CIMMYT (International Maize and Wheat Improvement Center), Mexico, D.F., Mexico.

Sarova, J., Hanzalova, A., and Bartos, P. 2005. Races of *Pyrenophora tritici-repentis* in the Czech Republic. *Acta Agrobotanica* 58:73-78.

Shabeer, A., and Bockus, A. A. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. *Plant Dis.* 72:599-602.

Sharp, E. L., Sally, B. K., and McNeal, F. H.1976. Effect of *Pyrenophora* wheat leaf blight on the thousand-kernel weight of 30 spring wheat cultivars. *Plant Dis.* 60:1 35-138.

Singh, P. K., M. Mergoum, Ali, S., Adhikari, T. B., Elias, E. M., Anderson, J. A., Glover, K. D., and Berzonsky, W. A. 2006. Evaluation of elite wheat germplasm for resistance to tan spot. *Plant Dis.* 90:1320-1325.

Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press Co., New York. 538 pp.

CHAPTER 6

SUMMARY AND CONCLUSION

The study provided additional information for further understanding various aspects of *Ptr*-wheat host interaction. Tan spot caused by the fungus *Pyrenophora tritici-repentis* is an important foliar disease worldwide. The fungus has a wide host range that include barley, oat, rye, and many non-cereal grasses in addition to its economic host wheat. Alternative hosts can play a significant role in the disease epidemiology and variation in the pathogen virulence and make it more difficult to develop economical and durable disease management strategies. Eight races have been identified in the fungal population and susceptibility to each race in the host is controlled by independent resistant gene. Tan spot was observed in the 1930's but it was not considered a major disease until the early 1970's. The widespread increase in tan spot incidence and severity in the 1970's and thereafter is anecdotally thought to be due to accidentally incorporation of tan spot susceptibility during the development of stem rust resistance cultivars to fight back this menace in the 1950' and 1960's. It may also be due to increase in the pathogen virulence due to its acquisition of *Ptr ToxA* gene from *Stagnospora nodorum*. In this study, we addressed this scenario of tan spot increase in the 1970's and beyond.

The results of this study based on evaluation of global collection 247 wheat genotypes mostly developed pre-stem rust epidemics against tan spot race 1, Ptr ToxA, and stem rust at seedlings in greenhouse. We observed 8 types of reactions in the evaluated wheat genotypes and that include 1) stem rust resistance and tan spot

resistance; 2) stem rust resistance and tan spot susceptible; 3) stem rust susceptible and tan spot resistance; 4) stem rust susceptible and tan spot susceptible; 5) tan spot resistance and Ptr ToxA insensitive; 6) tan spot resistance and Ptr ToxA sensitive; 7) tan spot susceptible and Ptr ToxA insensitive; and 8) tan spot susceptible and Ptr ToxA sensitive. It indicates a week or no correlation was observed between stem rust resistant cultivars - tan spot susceptibility and Ptr ToxA and tan spot susceptibility.

Rye is grown as forage and a rotational crop in South Dakota and also serves as an alternative host to *P. tritici-reentis*. Further it has been utilized as sources of resistance for diseases and pests tremendously in the improvement of modern wheat. We characterized 103 *P. tritici-repentis* isolates recovered from rye in South Dakota for their race structure. Additionally, we screened global collection of 211 rye genotypes and 8 commercial rye cultivars grown in the region against race 1, race 5, and Ptr ToxA. Majority (n=80) of the evaluated isolates lacks in *Ptr ToxA* gene and did not produce either necrosis or chlosrosis symptoms on tan spot wheat differential genotypes, hence identified as race 4. *Ptr ToxA* gene was amplified only in 23 isolates, which were grouped as race 1 based on their reaction on tan spot differential genotypes. Of 211 rye genotypes, 21% and 39% were susceptible to race 1 and race 5, respectively. Further, all rye genotypes exhibit susceptibility to race 1 and race 5 were insensitive to Ptr ToxA and Ptr ToxB toxins, respectively. Some of the rye cultivars planted in the region showed susceptibility to race 5. The results indicate that rye do not carry diverse *P. tritici-repentis* population prevalent in the state and may not have any significant role in tan

spot epidemiology. However, planting race 5 susceptible cultivars in the region may help in establishing race 5 population in the region.

Also, in our study we have characterized 569 *P. tritici-repentis* isolates recovered from wheat during 2012, 2013, and 2014 wheat growing seasons from South Dakota and evaluated for genotypic and phenotypic reaction to understand its race structure. Of 569 isolates, 510 amplified DNA bands of Ptr ToxA which may potentially be of race 1 and race 2; only 2 isolates were found to be with *Ptr ToxB* gene that may be of race 5 and race 6. Rest (n=57) of the isolates did not amplify any of the two toxins genes and they potentially be of race 3, race 4 or may be with a new virulence depending on their phenotypic reaction on tan spot differentials set. A random subset of 143 of 569 isolates genotyped for Ptr ToxA and Ptr ToxB genes were further phenotyped by inoculating them individually on tan spot wheat differential set. Of these 143 isolates, majority (n=100) were identified as race 1; one isolate was as race 2, 25 were as race 4, and only 2 isolates were identified as race 5. Six of 143 isolate that lacked in *Ptr ToxA* gene but behaved like race 2 as they induced necrosis on Ptr ToxA sensitive genotype Glenlea and did not produce any symptoms on the other three differential genotypes. These isolates may harbor additional necrosis inducing virulence genes as they lack in *Ptr ToxA* gene responsible for necrosis on Glenlea. Further, we evaluated 45 wheat cultivars grown in the region against tan spot race 1 and race 5. Nearly half of the spring wheat cultivars (n=24) exhibited susceptible to moderately susceptible reaction to both races. Six spring wheat cultivars were susceptible to both races. The results indicate that a diverse population of *P. tritici-repentis* exists on wheat in South Dakota. Race 5 was observed

for the first time in South Dakota. Wheat cultivars susceptible to race 5 further needed to be monitored for their role in race 5 spread.

Additionally, we performed genotypic and phenotypic race characterization of a large collection of *P. tritici-repentis* isolates recovered from wheat from Latvia, Lithuania, and Romania. We characterized 231 isolates collected from the three countries for *Ptr ToxA* and *Ptr ToxB* genes using two genes specific primers. Further, a subset of 139 of 231 isolates was characterized for their race structure by testing them on a tan spot wheat differentials set. Majority (83%) of the isolates amplified for *Ptr ToxA* gene suggesting prevalence of race 1 and 2. Further phenotypic characterization of 139 isolates also showed wide prevalence of race 1. Eighteen of the isolates did not seem to be any of the eight known races as they lacked *Ptr ToxA* gene but they behaved like either race 1 or race 2, suggesting possibility of novel toxins and emergence of new race structure might exist in Baltic region of these isolates as their virulence tools.

Based on the results obtained in this study indicate that widespread increase of tan spot in North America in the 1970's may not just be associated with deployment of stem rust resistant cultivars and/or increase in the pathogen virulence due acquisition of *Ptr ToxA* gene. A diverse *P. tritici-repentis* population exists in Baltic States and South Dakota. Further rye potentially does not have any role in the pathogen diversity in the region. Prevalence of the pathogen diverse population on wheat in the region warrants that regional wheat breeding programs should screen germplasm against all prevalent races in the development of tan spot resistance cultivars. Further, race 5 susceptible

cultivars planted in the region should be periodically monitored to assess any increase in race 5 population.

APPENDIX

Table 2.1. Disease reaction of 247 stem rust pre-epidemic bread wheat genotypes (156) and Durum genotypes (91) to host-selective toxin Ptr ToxA, race 1, and stem rust

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
1	CItr3986	*Triticum aestivum*	Algeria	1912	+	1.6	MR	S
2	CItr6868	*Triticum aestivum*	Argentina	1923	-	3.1	MS	S
3	CItr3968	*Triticum aestivum*	Argentina	1923	+	3.9	MS	S
4	CItr5100	*Triticum aestivum*	Argentina	na	+	3.3	MS	S
5	CItr8397	*Triticum aestivum*	Argentina	1926	-	1.7	MR	S
6	CItr2824	*Triticum aestivum*	Australia	1916	+	4.0	S	S
7	CItr5117	*Triticum aestivum*	Australia	1904	+	3.6	MS	S
8	CItr5125	*Triticum aestivum*	Australia	na	+	4.1	S	S
9	CItr6620	*Triticum aestivum*	Australia	1921	+	3.9	MS	S
10	CItr4608	*Triticum aestivum*	Australia	1915	+	4.5	S	S
11	CItr2826	*Triticum aestivum*	Australia	na	-	4.0	S	S
12	CItr8425	*Triticum aestivum*	Australia	1926	+	4.1	S	S
13	CItr8426	*Triticum aestivum*	Australia	1926	+	4.1	S	S
14	CItr8168	*Triticum aestivum*	Chile	1924	-	3.8	MS	S
15	CItr8111	*Triticum aestivum*	China	1923	-	3.7	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
16	CItr8116	*Triticum aestivum*	China	1924	+	3.8	MS	S
17	CItr8117	*Triticum aestivum*	China	1924	+	3.6	MS	S
18	CItr5085	*Triticum aestivum*	China	1916	-	1.8	MR	S
19	CItr5079	*Triticum aestivum*	China	1924	-	3.5	MS	S
20	CItr8328	*Triticum aestivum*	China	1925	-	2.2	MR	S
21	CItr8332	*Triticum aestivum*	China	1925	+	4.3	MS	S
22	CItr8333	*Triticum aestivum*	China	1916	-	3.5	MS	S
23	CItr8128	*Triticum aestivum*	Ethiopia	1924	-	1.9	MR	S
24	CItr7952	*Triticum aestivum*	Ethiopia	1924	+	1.7	MR	R
25	CItr7967	*Triticum aestivum*	Ethiopia	1924	-	1.2	R	S
26	CItr8074	*Triticum aestivum*	Ethiopia	1924	+	1.6	MR	S
27	CItr8082	*Triticum aestivum*	Ethiopia	1924	+	3.5	MS	S
28	CItr7808	*Triticum aestivum*	Ethiopia	1924	-	4.1	S	S
29	CItr7858	*Triticum aestivum*	Ethiopia	1924	-	3.9	S	S
30	CItr7877	*Triticum aestivum*	Ethiopia	1924	+	4	S	S
31	CItr5018	*Triticum aestivum*	Former Soviet Union	1916	-	3.6	MS	S
32	CItr5878	*Triticum aestivum*	Former Soviet Union	1917	-	3.7	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
33	CItr2346	*Triticum aestivum*	France	1903	-	2	MR	S
34	CItr2440	*Triticum aestivum*	Germany	1904	-	1.9	MR	S
35	CItr5111	*Triticum aestivum*	Greece	1916	-	2	MR	S
36	CItr4203	*Triticum aestivum*	Honduras	1914	-	3.9	S	S
37	CItr2034	*Triticum aestivum*	Hungary	1900	-	1	R	S
38	CItr4302	*Triticum aestivum*	Iran	1914	+	4.3	S	S
39	CItr4305	*Triticum aestivum*	Iran	1914	-	3.8	MS	S
40	CItr4309	*Triticum aestivum*	Iran	1914	-	3.8	MS	S
41	CItr4312	*Triticum aestivum*	Iran	1914	+	4	S	S
42	CItr8428	*Triticum aestivum*	Japan	1926	-	3.5	MS	S
43	CItr8433	*Triticum aestivum*	Japan	1926	-	4	S	S
44	CItr6833	*Triticum aestivum*	Kenya	1922	-	1.5	R	S
45	CItr2796	*Triticum aestivum*	Mexico	1904	-	1	R	S
46	CItr5286	*Triticum aestivum*	Mexico	1926	-	1.7	MR	S
47	CItr5288	*Triticum aestivum*	Mexico	1922	-	3.8	MS	S
48	CItr8400	*Triticum aestivum*	Mexico	1926	-	4.1	S	S
49	CItr8401	*Triticum aestivum*	Mexico	1926	-	1.7	MR	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
50	CItr8402	*Triticum aestivum*	Mexico	1926	+	4.2	S	S
51	CItr8408	*Triticum aestivum*	Mexico	1926	-	2	MR	S
52	CItr8409	*Triticum aestivum*	Mexico	1926	-	3.8	MS	S
53	CItr8415	*Triticum aestivum*	Mexico	1926	-	4.3	S	S
54	CItr8417	*Triticum aestivum*	Mexico	1926	-	4	S	S
55	CItr8418	*Triticum aestivum*	Mexico	1926	-	1.3	R	S
56	CItr8429	*Triticum aestivum*	Mexico	1926	-	3.6	MS	S
57	CItr6877	*Triticum aestivum*	Morocco	1923	-	1.5	R	S
58	CItr7737	*Triticum aestivum*	Pakistan	1923	-	3.5	MS	S
59	CItr8161	*Triticum aestivum*	Peru	1924	-	1.7	MR	S
60	CItr4175	*Triticum aestivum*	Philippines	1914	-	4	S	S
61	CItr7715	*Triticum aestivum*	Russian Federation	1915	+	3.2	MS	S
62	CItr7717	*Triticum aestivum*	Russian Federation	1923	-	4	S	S
63	CItr7720	*Triticum aestivum*	Russian Federation	1923	+	1.8	MR	S
64	CItr7725	*Triticum aestivum*	Russian Federation	1923	+	2	MR	S
65	CItr7576	*Triticum aestivum*	Russian Federation	1923	+	1.7	MR	S
66	CItr7700	*Triticum aestivum*	Russian Federation	1923	-	1.9	MR	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
67	CItr7708	*Triticum aestivum*	Russian Federation	1923	-	1.9	MR	S
68	CItr5015	*Triticum aestivum*	Russian Federation	1923	-	1.7	MR	S
69	CItr3733	*Triticum aestivum*	Russian Federation	1916	-	2.5	MR	S
70	CItr3737	*Triticum aestivum*	Russian Federation	1913	-	3.8	S	S
71	CItr3756	*Triticum aestivum*	Russian Federation	1912	-	1.7	S	S
72	CItr4320	*Triticum aestivum*	Russian Federation	1912	+	3.9	S	S
73	CItr4322	*Triticum aestivum*	Russian Federation	1915	+	3.8	MS	S
74	CItr4795	*Triticum aestivum*	Russian Federation	1916	-	2.4	MS	S
75	CItr4795	*Triticum aestivum*	South Africa	1924	-	1.7	MR	S
76	CItr3008	*Triticum aestivum*	South Africa	1906	+	4.4	S	S
77	CItr3285	*Triticum aestivum*	Sweden	1912	-	1.2	R	S
78	CItr6009	*Triticum aestivum*	Sweden	1917	+	2	MR	S
79	CItr6873	*Triticum aestivum*	Tunisia	1923	-	4	S	S
80	CItr3812	*Triticum aestivum*	Tunisia	1912	-	2.2	MR	S
81	CItr3814	*Triticum aestivum*	Tunisia	1913	-	1.7	MR	S
82	CItr6791	*Triticum aestivum*	United Kingdom	1922	-	2.1	MR	S
83	CItr6886	*Triticum aestivum*	USA	1923	-	3.5	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
84	CItr6887	*Triticum aestivum*	USA	1923	+	3.7	MS	S
85	CItr4966	*Triticum aestivum*	USA	1915	-	1.7	MR	S
86	CItr5149	*Triticum aestivum*	USA	1916	-	1.7	MR	S
87	CItr4475	*Triticum aestivum*	USA	1915	-	1.3	R	S
88	CItr8215	*Triticum aestivum*	USA	1926	-	2.2	MR	S
89	CItr8216	*Triticum aestivum*	USA	1926	-	4	S	S
90	CItr8873	*Triticum aestivum*	USA	1928	+	3.3	MS	S
91	CItr11789	*Triticum aestivum*	USA	1936	+	3.8	S	S
92	CItr3690	*Triticum aestivum*	USA	1913	-	1.5	R	S
93	CItr5296	*Triticum aestivum*	USA	1916	+	1.7	MR	S
94	CItr12039	*Triticum aestivum*	USA	1940	-	1.5	R	S
95	CItr11684	*Triticum aestivum*	USA	na	+	3.9	S	S
96	CItr11685	*Triticum aestivum*	USA	na	-	2.1	MR	S
97	CItr12038	*Triticum aestivum*	USA	1940	+	4	S	S
98	CItr6900	*Triticum aestivum*	USA	1923	+	3.5	MS	S
99	CItr8004	*Triticum aestivum*	USA	1925	-	4	S	S
100	CItr6049	*Triticum aestivum*	USA	1918	-	2.8	MR	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
101	CItr8385	*Triticum aestivum*	USA	1927	-	3.5	MS	S
102	CItr2874	*Triticum aestivum*	USA	1905	-	3.8	MS	S
103	CItr8199	*Triticum aestivum*	USA	1926	-	4	S	S
104	CItr8379	*Triticum aestivum*	USA	na	-	2.7	MR	S
105	CItr2874	*Triticum aestivum*	USA	1927	-	1.7	MR	S
106	CItr8384	*Triticum aestivum*	USA	1927	+	4	S	S
107	CItr11642	*Triticum aestivum*	USA	1934	+	3.9	S	S
108	CItr11683	*Triticum aestivum*	USA	1935	+	3.8	MS	S
109	CItr11713	*Triticum aestivum*	USA	1935	+	1.9	MR	S
110	CItr11714	*Triticum aestivum*	USA	1935	+	3.5	MS	S
111	CItr11715	*Triticum aestivum*	USA	1935	-	2.3	MR	S
112	CItr11716	*Triticum aestivum*	USA	1935	+	2.2	MR	S
113	CItr11723	*Triticum aestivum*	USA	1935	+	4.3	S	S
114	CItr8879	*Triticum aestivum*	USA	1928	+	4	S	S
115	CItr8890	*Triticum aestivum*	USA	1929	+	4.1	S	S
116	CItr12056	*Triticum aestivum*	USA	1940	+	3.8	MS	S
117	CItr12057	*Triticum aestivum*	USA	1940	+	3.9	S	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
118	CItr12060	*Triticum aestivum*	USA	1940	-	2.3	MR	S
119	CItr12204	*Triticum aestivum*	USA	1941	+	2.8	MR	S
120	CItr11775	*Triticum aestivum*	USA	1935	+	4	S	S
121	CItr11779	*Triticum aestivum*	USA	1935	+	4.1	S	S
122	CItr11786	*Triticum aestivum*	USA	1936	+	4	S	S
123	CItr11787	*Triticum aestivum*	USA	1936	+	3.5	MS	R
124	CItr11788	*Triticum aestivum*	USA	1936	-	3.5	MS	S
125	CItr10014	*Triticum aestivum*	USA	1930	+	4	S	S
126	CItr11479	*Triticum aestivum*	USA	1932	+	1.7	MR	S
127	CItr11550	*Triticum aestivum*	USA	1932	+	3.9	S	S
128	CItr11639	*Triticum aestivum*	USA	1934	+	3.5	MS	S
129	CItr11640	*Triticum aestivum*	USA	1934	+	3.6	MS	S
130	CItr11641	*Triticum aestivum*	USA	1934	+	4	S	S
131	CItr12045	*Triticum aestivum*	USA	1940	+	2	MR	S
132	CItr12046	*Triticum aestivum*	USA	1940	+	3.5	MS	S
133	CItr12048	*Triticum aestivum*	USA	1940	+	4	S	S
134	CItr12049	*Triticum aestivum*	USA	1940	+	3.8	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
135	CItr12050	*Triticum aestivum*	USA	1940	+	4	S	S
136	CItr12051	*Triticum aestivum*	USA	1940	+	3.8	MS	S
137	CItr12052	*Triticum aestivum*	USA	1940	+	4	S	S
138	CItr12053	*Triticum aestivum*	USA	1940	-	4.1	S	S
139	CItr12054	*Triticum aestivum*	USA	1940	+	3.5	S	S
140	CItr12055	*Triticum aestivum*	USA	1940	+	3.8	MS	S
141	CItr11798	*Triticum aestivum*	USA	1936	+	3.7	S	S
142	CItr11799	*Triticum aestivum*	USA	1936	+	3.8	MS	S
143	CItr11882	*Triticum aestivum*	USA	1937	+	1.8	MR	S
144	CItr11883	*Triticum aestivum*	USA	1937	+	3.7	MS	S
145	CItr11884	*Triticum aestivum*	USA	na	+	1.9	MR	S
146	CItr11885	*Triticum aestivum*	USA	1937	+	1.7	MR	S
147	CItr11894	*Triticum aestivum*	USA	1938	+	3.8	MS	S
148	CItr11895	*Triticum aestivum*	USA	1938	+	3.8	MS	S
149	CItr11899	*Triticum aestivum*	USA	1939	+	4	S	S
150	CItr11930	*Triticum aestivum*	USA	1938	+	4	S	S
151	CItr11931	*Triticum aestivum*	USA	1938	+	3.7	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
152	CItr11934	*Triticum aestivum*	USA	1939	+	3.7	MS	S
153	CItr11948	*Triticum aestivum*	USA	1939	+	4	S	S
154	CItr11949	*Triticum aestivum*	USA	1939	+	4.1	S	S
155	CItr12005	*Triticum aestivum*	USA	1939	+	2	MR	S
156	CItr12037	*Triticum aestivum*	USA	1940	+	3.6	MS	S
157	CItr15055	*Triticum turgidum*	Afghanistan	1970	-	4	S	S
158	CItr15065	*Triticum turgidum*	Afghanistan	1970	-	4.2	S	S
159	CItr3847	*Triticum turgidum*	Algeria	1912	+	3.8	MS	S
160	CItr6834	*Triticum turgidum*	Algeria	1922	-	4.8	S	S
161	CItr1471	*Triticum turgidum*	Algeria	1900	-	4.3	S	S
162	CItr3848	*Triticum turgidum*	Algeria	1912	-	4	S	S
163	CItr3856	*Triticum turgidum*	Algeria	1912	-	4.1	S	S
164	PI174673	*Triticum turgidum*	Algeria	1949	+	3.8	MS	R
165	PI184538	*Triticum turgidum*	Algeria	1949	-	4	S	S
166	PI 7794	*Triticum turgidum*	Algeria	1900	+	2.8	MR	S
167	PI 7796	*Triticum turgidum*	Algeria	1901	+	2.3	MR	S
168	PI 25987	*Triticum turgidum*	Algeria	1909	+	4.1	S	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
169	PI57069	*Triticum turgidum*	Algeria	1923	+	3.4	MS	S
170	PI 174624	*Triticum turgidum*	Algeria	1949	-	1.3	R	S
171	PI94723	*Triticum turgidum*	Asia Minor	1931	+	3.4	MS	S
172	CItr5122	*Triticum turgidum*	Australia	1916	+	4.2	S	S
173	CItr13771	*Triticum turgidum*	Canada	1963	+	2	MR	R
174	CItr17757	*Triticum turgidum*	Canada	1978	-	4.1	S	S
175	CItr11246	*Triticum turgidum*	Croatia	1929	+	4.2	S	S
176	CItr7800	*Triticum turgidum*	Ethiopia	1924	+	4.3	S	R
177	CItr7780	*Triticum turgidum*	Ethiopia	1924	+	4.1	S	S
178	CItr7800	*Triticum turgidum*	Ethiopia	1924	+	4.3	S	R
179	CItr8635	*Triticum turgidum*	Ethiopia	1926	+	3.7	MS	S
180	CItr14712	*Triticum turgidum*	Ethiopia	1970	+	4	S	R
181	CItr3322	*Triticum turgidum*	Former Soviet Union	1912	-	3.6	MS	S
182	CItr15159	*Triticum turgidum*	France	1971	+	4.1	S	S
183	CI7157	*Triticum turgidum*	Georgia	1923	-	3.8	S	S
184	CItr2462	*Triticum turgidum*	Germany	1904	-	3.7	MS	S
185	CItr8373	*Triticum turgidum*	India	1927	+	4.2	S	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
186	CItr9032	*Triticum turgidum*	Iraq	1926	-	1.9	MR	S
187	CItr12452	*Triticum turgidum*	Italy	1946	-	4.2	S	S
188	CItr15111	*Triticum turgidum*	Italy	1971	-	4.3	S	S
189	CItr2800	*Triticum turgidum*	Mexico	1904	-	4.3	S	R
190	CItr6878	*Triticum turgidum*	Morocco	1923	-	4.3	S	S
191	CItr6879	*Triticum turgidum*	Morocco	1923	-	4.2	S	S
192	CItr8447	*Triticum turgidum*	Morocco	1926	-	2.1	MR	S
193	PI94702	*Triticum turgidum*	Palestine	1931	-	3.5	S	S
194	CItr8163	*Triticum turgidum*	Peru	1924	+	4.4	S	R
195	CItr8164	*Triticum turgidum*	Peru	1924	-	4.1	S	S
196	CItr2793	*Triticum turgidum*	Portugal	1904	-	1	R	S
197	CItr1515	*Triticum turgidum*	Russian Federation	1900	-	1.8	MR	S
198	CItr11220	*Triticum turgidum*	Serbia	1929	-	4.1	S	S
199	PI193391	*Triticum turgidum*	Syria	1951	+	4.6	S	S
200	PI 166984	*Triticum turgidum*	Syria	1948	-	2	MR	S
201	CItr3198	*Triticum turgidum*	Tunisia	1911	-	1	R	S
202	CItr3199	*Triticum turgidum*	Tunisia	1911	-	4	S	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
203	CItr3200	*Triticum turgidum*	Tunisia	1911	-	4	S	S
204	CItr3207	*Triticum turgidum*	Tunisia	1911	+	4.5	S	S
205	CItr3209	*Triticum turgidum*	Tunisia	1911	+	4.1	S	S
206	CItr3211	*Triticum turgidum*	Tunisia	1911	+	4	S	S
207	CItr3221	*Triticum turgidum*	Tunisia	1911	+	3.5	MS	S
208	CItr3233	*Triticum turgidum*	Tunisia	1911	-	4.3	S	S
209	CItr3242	*Triticum turgidum*	Tunisia	1911	-	3.8	MS	S
210	CItr3243	*Triticum turgidum*	Tunisia	1911	+	4.3	S	S
211	CItr3260	*Triticum turgidum*	Tunisia	1911	-	4	S	S
212	CItr3262	*Triticum turgidum*	Tunisia	1911	+	1.3	R	S
213	CItr3798	*Triticum turgidum*	Tunisia	1912	+	4.3	S	S
214	CItr3140	*Triticum turgidum*	Tunisia	1911	+	4.2	S	S
215	CItr3146	*Triticum turgidum*	Tunisia	1911	+	3.7	MS	S
216	CItr3156	*Triticum turgidum*	Tunisia	1911	-	3.8	MS	na
217	CItr3169	*Triticum turgidum*	Tunisia	1911	-	4	S	S
218	CItr3187	*Triticum turgidum*	Tunisia	1911	+	3.9	S	S
219	CItr3193	*Triticum turgidum*	Tunisia	1911	+	3.7	MS	S

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
220	CItr3195	*Triticum turgidum*	Tunisia	1911	-	1.7	MR	S
221	CItr3809	*Triticum turgidum*	Tunisia	1912	-	4.1	S	S
222	CItr15483	*Triticum turgidum*	Tunisia	1912	+	4.2	S	S
223	CItr15415	*Triticum turgidum*	Tunisia	1972	+	4.1	S	R
224	CItr15418	*Triticum turgidum*	Tunisia	1971	-	2.7	MR	S
225	CItr15479	*Triticum turgidum*	Tunisia	1972	+	2.6	MR	S
226	CItr3811	*Triticum turgidum*	Tunisia	1912	+	4.1	S	S
227	CItr15413	*Triticum turgidum*	Tunisia	1972	+	1.9	MR	S
228	CItr5414	*Triticum turgidum*	Tunisia	1972	-	4.2	S	S
229	PI 166941	*Triticum turgidum*	Turkey	1948	-	4.1	S	S
230	CItr13337	*Triticum turgidum*	USA	1957	+	3.7	MS	S
231	CItr13758	*Triticum turgidum*	USA	1962	+	1.8	MR	R
232	CItr14041	*Triticum turgidum*	USA	1964	+	3.7	MS	R
233	CItr14042	*Triticum turgidum*	USA	1964	+	3.5	S	R
234	CItr14043	*Triticum turgidum*	USA	1964	+	1.8	MR	R
235	CItr14044	*Triticum turgidum*	USA	1964	+	3.8	S	R
236	CItr14045	*Triticum turgidum*	USA	1964	+	4.1	S	R

Table 2.1. continued from previous page

No.	Accession #	Wheat type	Country	Year	Ptr ToxA	Lesion type	Race 1	Stem rust
237	CItr14046	*Triticum turgidum*	USA	1964	+	1.8	MR	R
238	CItr11776	*Triticum turgidum*	USA	1964	+	4.3	S	S
239	GSTR 10902	*Triticum turgidum*	USA	2008	+	3.9	S	S
240	GSTR 10906	*Triticum turgidum*	USA	2008	+	1.9	MR	S
241	GSTR 10915	*Triticum turgidum*	USA	2008	+	3.5	MS	R
242	GSTR 10916	*Triticum turgidum*	USA	2008	+	4	S	R
243	GSTR 10917	*Triticum turgidum*	USA	2008	+	3.3	MS	S
244	GSTR 10918	*Triticum turgidum*	USA	2008	-	4	S	S
245	GSTR 10919	*Triticum turgidum*	USA	2008	+	4	S	R
246	GSTR 10922	*Triticum turgidum*	USA	2008	-	1.7	MR	S
247	GSTR 10943	*Triticum turgidum*	USA	2008	+	4	S	R

Plants were rated on a 1 to 5 lesion type rating scale (Lamari et al. 1989a) with 1-2 is resistant(R) to moderately resistant(MR), 3 is moderately susceptible(MS) and 4-5 are susceptible(S) with necrotic symptoms. Reactions to toxin/culture filtrate were scored as + and –, indicating sensitivity and insensitivity, respectively. Toxin Ptr ToxA is produced by race 1. For stem rust ITs 0, 1, 2, or any combination of these indicated resistant(R), and ITs 3 or 4 indicated susceptible(S) according to system (Stakman et al.1962)

Table 3.1. List of 231 *P. tritici-repentis* isolates received from Latvia (LV), Lithuania (LT), Romania (Ro) and analyzed for their phenotypic and genotypic race characterization

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
1	13-LT-1-1	1	+	-	+
2	13-LT-2-1	1	+	-	+
3	13-LT-3-1	1	+	-	+
4	13-LT-4-1	1	+	-	+
5	13-LT-5-1	1	+	-	+
6	13-LT-1-2	1	+	-	+
7	13-LT-2-2	1	+	-	+
8	13-LT-3-2	*Nec+chl+*	-	-	+
9	13-LT-4-3	1	+	-	+
10	13-LT-5-3	1	+	-	+
11	13-LT-6-3	3	-	-	+
12	13-LT-1-4	1	+	-	+
13	13-LT-1-3	1	+	-	+
14	13-LT-2-3	1	+	-	+
15	13-LT-3-3	1	+	-	+
16	13-LT-2-4	1	+	-	+
17	13-LT-3-4	1	+	-	+
18	13-LT-4-4	1	+	-	+
19	13-LT-5-4	1	+	-	+
20	13-LT-6-4	*Nec+chl+*	-	-	+
21	13-LT-1-13	1	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
22	13-LT-2-5	1	+	-	+
23	13-LT-2-15	*Nec+chl+*	-	-	+
24	13-LT-3-5	1	+	-	+
25	13-LT-4-5	1	+	-	+
26	13-LT-5-5	1	+	-	+
27	13-LT-6-5	3	-	-	+
28	13-LT-1-5	1	+	-	+
29	13-LT-1-6	1	+	-	+
30	13-LT-1-7	1	+	-	+
31	13-LT-1-8	3	+	-	+
32	13-LT-1-9	1	+	-	+
33	13LT-1-10	1	+	-	+
34	13-LT-1-11	2	+	-	+
35	13-LT-1-12	3	-	-	+
36	13-LT-1-13	1	+	-	+
37	13-LT-1-14	1	+	-	+
38	13-LT-2-6	1	+	-	+
39	13-LT-2-7	1	+	-	+
40	13-LT-2-8	4	-	-	+
41	13-LT-2-9	*	+	-	+
42	13-LT-2-10	*Nec+chl+*	-	-	+
43	13-LT-2-11	3	-	-	+
44	13-LT-2-12	*Nec+chl+*	-	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
45	13-LT-2-13	1	+	-	+
46	13-LT-2-14	*Nec+chl+*	-	-	+
47	13-LT-2-15	*Nec+chl+*	-	-	+
48	13-LT-2-16	*Nec+chl+*	-	-	+
49	13-LT-3-6	3	-	-	+
50	13-LT-3-7	3	-	-	+
51	13-LT-3-8	*	+	-	+
52	13-LT-3-9	*Nec+chl+*	-	-	+
53	13-LT-3-10	*	+	-	+
54	13-LT-3-11	*Nec+chl+*	-	-	+
55	13-LT-3-12	*Nec+chl+*	-	-	+
56	13-LT-3-13	*	+	-	+
57	13-LT-3-14	3	-	-	+
58	13-LT-3-15	4	-	-	+
59	13-LT-3-16	*	+	-	+
60	13-LT-3-17	1	+	-	+
61	13-LT-3-18	*	+	-	+
62	13-LT-3-19	*	+	-	+
63	13-LT-3-20	*	+	-	+
64	13-LT-3-21	3	-	-	+
65	13-LT-3-22	*	+	-	+
66	13-LT-3-23	3	-	-	+
67	13-LT-3-24	*	+	-	+
68	13-LT-3-25	3	-	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
69	13-LT4-2	4	-	-	+
70	13-LT4-6	3	-	-	+
71	13-LT4-7	1	+	-	+
72	13-LT4-8	*	+	-	+
73	13-LT4-9	*Nec+chl+*	-	-	+
74	13-LT4-10	*Nec+chl+*	-	-	+
75	13-LT4-11	*Nec+chl+*	-	-	+
76	13-LT-4-12	*	+	-	+
77	13-LT-4-13	*	+	-	+
78	13-LT-4-14	*Nec+chl+*	-	-	+
79	13-LT-4-15	*Nec+chl+*	-	-	+
80	13-LT-5-2	*	+	-	+
81	13-LT-5-6	1	+	-	+
82	13-LT-5-7	*	+	-	+
83	13-LT-5-8	*	+	-	+
84	13-LT-5-9	1	+	-	+
85	13-LT-5-10	*	+	-	+
86	13-LT-5-11	*	+	-	+
87	13-LT-5-12	*	+	-	+
88	13-LT-5-13	*	+	-	+
89	13-LT-5-14	*Nec+chl+*	-	-	+
90	13-LT-5-15	*	+	-	+
91	13-LT-6-2	*	+	-	+
92	13-LT-4-11	*	-	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
93	13-LT-4-12	3	-	-	+
94	13-LT-4-13	*	+	-	+
95	13-LT-4-14	4	-	-	+
96	13-LT-4-15	4	-	-	+
97	13-LT-5-2	*	+	-	+
98	13-LT-6-6	*	+	-	+
99	13-LT-6-7	4	-	-	+
100	13-LT-6-8	*	+	-	+
101	13-LT-6-9	*	+	-	+
102	13-LT-6-10	*	+	-	+
103	13-LT-6-11	3	-	-	+
104	13-LT-6-12	*	+	-	+
105	13-LT-6-13	*	+	-	+
106	13-LT-6-14	*	+	-	+
107	13-LT-6-15	3	-	-	+
108	14-Ro-1-1	1	+	-	+
109	14-Ro-1-2	2	+	-	+
110	14-Ro-1-3	1	+	-	+
111	14-Ro-1-4	1	+	-	+
112	14-Ro-1-5	1	+	-	+
113	14-Ro-1-6	*	+	-	+
114	14-Ro-1-7	1	+	-	+
115	14-Ro-1-8	2	+	-	+
116	14-Ro-1-9	1	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
117	14-Ro- 1-10	1	+	-	+
118	14-Ro-1-11	2	+	-	+
119	14-Ro-1-12	*	+	-	+
120	14-Ro-1-13	*	+	-	+
121	14-Ro-1-14	*	+	-	+
122	14-Ro-1-15	*	+	-	+
123	14-Ro-1-16	*	+	-	+
124	14-Ro-1-17	*	+	-	+
125	14-Ro-1-18	*	+	-	+
126	14-Ro-1-19	*	+	-	+
127	14-Ro-1-20	*	+	-	+
128	14-Ro-2-1	*	+	-	+
129	14-Ro-2-2	*	+	-	+
130	14-Ro-2-3	1	+	-	+
131	14-Ro-2-4	1	+	-	+
132	14-Ro-2-6	1	+	-	+
133	14-Ro-2-7	*	+	-	+
134	14-Ro-2-8	*	+	-	+
135	14-Ro-2-9	*	+	-	+
136	14-Ro-2-10	*	+	-	+
137	14-Ro-2-11	*	+	-	+
138	14-Ro-2-12	*	+	-	+
139	14-Ro-3-1	*	+	-	+
140	14-Ro-3-2	1	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
141	14-Ro-3-3	1	+	-	+
142	14-Ro-3-4	1	+	-	+
143	14-Ro-3-5	*	+	-	+
144	14-Ro-3-6	1	+	-	+
145	14-Ro-3-7	2	+	-	+
146	14-Ro-3-8	1	+	-	+
147	14-Ro-3-9	*	+	-	+
148	14-Ro-3-10	*	+	-	+
149	14-Ro-3-11	1	+	-	+
150	14-Ro-3-13	2	+	-	+
151	14-Ro-3-14	*	+	-	+
152	14-Ro-3-15	*	+	-	+
153	14-Ro-3-16	*	+	-	+
154	14-Ro-3-17	*	+	-	+
155	14-Ro-3-18	*	+	-	+
156	14-Ro-3-19	*	+	-	+
157	14-Ro-3-20	*	+	-	+
158	14-Ro-3-21	*	+	-	+
159	14-Ro-3-22	*	+	-	+
160	14-Ro-3-23	*	+	-	+
161	14-Ro-3-24	*	+	-	+
162	14-Ro-3-25	*	+	-	+
163	14-Ro-3-26	1	+	-	+
164	14-Ro-3-27	*	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
165	14-Ro-3-28	*	+	-	+
166	14-Ro-3-29	*	+	-	+
167	14-Ro-3-30	*	+	-	+
168	14-Ro-4-1	2	+	-	+
169	14-Ro-4-2	1	+	-	+
170	14-Ro-4-3	1	+	-	+
171	14-Ro-4-4	*	+	-	+
172	14-Ro-5-1	*	+	-	+
173	14-Ro-5-2	1	+	-	+
174	14-Ro-5-4	1	+	-	+
175	14-Ro-5-5	1	+	-	+
176	14-Ro-5-6	1	+	-	+
177	14-Ro-5-7	1	+	-	+
178	14-Ro-5-8	1	+	-	+
179	14-Ro-5-9	2	+	-	+
180	14-Ro-5-11	1	+	-	+
181	14-Ro-5-13	1	+	-	+
182	14-Ro-5-14	1	+	-	+
183	14-Ro-5-15	*	+	-	+
184	14-Ro-5-16	*	+	-	+
185	14-Ro-5-17	1	+	-	+
186	14-Ro-5-18	2	+	-	+
187	14-Ro-5-19	*	+	-	+
188	14-Ro-5-20	*	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
189	14-Ro-5-21	*	+	-	+
190	14-Ro-5-22	*	+	-	+
191	14-Ro-5-23	*Nec+chl-*	-	-	+
192	14-Ro-5-24	*	+	-	+
193	14-Ro-5-25	1	+	-	+
194	14-Ro-5-26	*	+	-	+
195	14-Ro-5-27	*	+	-	+
196	14-Ro-5-28	*	+	-	+
197	14-Ro-5-29	*	+	-	+
198	14-Ro-5-30	*	+	-	+
199	14-Ro-5-31	4	-	-	+
200	14-Ro-5-32	*	+	-	+
201	14-Ro-5-33	*	+	-	+
202	14-Ro-6-1	*	+	-	+
203	14-Ro-6-2	1	+	-	+
204	14-Ro-6-3	1	+	-	+
205	14-Ro-6-4	1	+	-	+
206	14-Ro-6-5	1	+	-	+
207	14-Ro-6-6	1	+	-	+
208	14-Ro-6-7	1	+	-	+
209	14-Ro-6-8	1	+	-	+
210	14-Ro-6-9	1	+	-	+
211	14-Ro-6-10	1	+	-	+
212	14-Ro-6-11	1	+	-	+

Table 3.1. continued from previous page

No	Isolate	Race	*Ptr ToxA*	*Ptr ToxB*	*MT*[a]
213	14-Ro-6-12	1	+	-	+
214	14-Ro-6-13	1	+	-	+
215	14-Ro-6-14	1	+	-	+
216	14-Ro-6-15	2	+	-	+
217	14-LV-1	*	+	-	+
218	14-LV-2	1	+	-	+
219	14-LV-3	1	+	-	+
220	14-LV-4	1	+	-	+
221	14-LV-5	1	+	-	+
222	14-LV-6	*	+	-	+
223	14-LV-7	*	+	-	+
224	14-LV-8	Race 1	+	-	+
225	14-LV-9	Race 1	+	-	+
226	14-LV-10	Race 1	+	-	+
227	14-LV-11	*	+	-	+
228	14-LV-12	Race 2	+	-	+
229	14-LV-13	Race 1	+	-	+
230	14-LV-14	*	+	-	+
231	14-LV-15	Race 1	+	-	+

Nec+Chl+ = Isolates lack in *Ptr ToxA* gene but produce necrosis on Ptr ToxA sensitive wheat differential Glenlea and chlorosis on Ptr ToxC sensitive differential 6B365; Nec+Chl- = Isolates lack in *Ptr ToxA* gene but produce necrosis on Ptr ToxA sensitive wheat differential Glenlea; [a] = *P. tritici-repentis* specific mating gene was amplified; * = Isolates were not race characterized on tan spot wheat differentials set; + = Ptr ToxA/ ToxB/mating type gene present and - = Ptr ToxA/ ToxB/mating type gene not present.

Table 4.1. Global collection of 211 rye (*Secale cereale subsp. Cereale*) tested against (tan spot) *Pyrenophora tritici-repentis* race 1 (n=211), race 5 (171)

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
1	Afghanistan	PI 223896	1	R	2	MR
2	Afghanistan	PI 275356	3	MS	2	MR
3	Afghanistan	PI 366503	4	S	2	MR
4	Afghanistan	PI 253957	3	MS	1	R
5	Algeria	PI 280842	2	MR	1	R
6	Argentina	PI 240676	1	R	1	R
7	Argentina	PI 534987	1	R	1	R
8	Argentina	PI 534988	1	R	4	S
9	Argentina	PI 543398	5	S	1	R
10	Argentina	PI 534951	0	0	2	MR
11	Argentina	PI 534988	0	0	4	S
12	Argentina	PI 535015	0	0	2	MR
13	Australia	Clse 79	2	MR	1	R
14	Australia	PI 345739	2	MR	1	R
15	Australia	PI 345740	2	MR	2	MR
16	Australia	PI 346416	2	MR	1	R
17	Austria	PI 254810	4	S	1	R
18	Austria	PI 534960	4	S	4	MS
19	Austria	PI 535007	1	R	4	S
20	Austria	PI535004	0	0	2	MR
21	Belarus	PI 372116	3	MS	1	R
22	Belarus	PI 372119	4	S	1	R
23	Belgium	PI 534970	3	MS	3	MS
24	Bosnia	PI 349919	3	MS	1	R
25	Bosnia	PI 349923	2	MR	1	R
26	Brazil	PI 239580	3	MS	1	R
27	Brazil	PI 241578	3	MS	1	R
28	Brazil	PI 314964	3	MS	1	R
29	Brazil	PI 542470	1	R	2	MR
30	Bulgaria	PI 294794	1	R	2	MR
31	Bulgaria	PI 294795	4	S	2	MR
32	Canada	PI 445984	3	MS	1	R
33	Canada	PI 445998	3	MS	2	MR
34	Canada	PI 496254	3	MS	1	R
35	Canada	PI 590948	3	MS	2	MR
36	Canada	PI 445999	0	0	1	R

Table 4.1. continued from previous page

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
37	Canada	PI 534946	0	0	3.5	MS
38	Canada	PI 534952	0	0	3	MS
39	Canada	PI 534968	0	0	3.5	MS
40	Canada	PI 535008	0	0	4	S
41	Canada	PI 496256	0	0	4	S
42	Chile	PI 436165	3	MS	1	R
43	Chile	PI 436171	4	S	3.5	MS
44	Chile	PI 436185	0	0	2	MR
45	Chile	PI 436192	0	0	4	S
46	China	PI 447337	3	MS	1	R
47	China	PI 452132	2	MR	3.5	MS
48	China	PI 452133	4	S	4	S
49	Czech Republic	PI 534956	2	MR	1	R
50	Estonia	PI 265471	1	R	1	R
51	Estonia	PI 372114	5	S	2	MR
52	Estonia	PI 446514	4	S	4	S
53	Finland	PI 265473	1	R	1	R
54	Finland	PI 290440	4	S	3	MS
55	France	PI 235536	3	MS	3	MS
56	France	PI 315957	3	MS	3.5	MS
57	France	PI 535144	1	R	1	R
58	France	PI 446136	0	0	1	R
59	Germany	PI 290435	3	MS	1	MS
60	Germany	PI 330424	4	S	2	MR
61	Germany	PI 534963	0	0	3	MS
62	Germany	PI 534969	0	0	2	MR
63	Germany	PI 534933	0	0	1	R
64	Greece	PI 249936	3	MS	1	R
65	Greece	PI 446151	2	MR	2	MR
66	Hungary	PI 272333	1	R	2.5	MR
67	Hungary	PI 290436	3	MS	2	MR
68	Hungary	PI 534989	0	0	3.5	MS
69	Hungary	PI 534928	0	0		MR
70	India	PI 430004	1	R	2	MR
71	Iran	PI 227870	1	R	2	MR
72	Iran	PI 243741	1	R	1	R
73	Iran	PI 250744	4	S	1	R
74	Iran	PI 250746	1	R	1	R

Table 4.1. continued from previous page

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
75	Iran	PI 289814	2	MR	2	MR
76	Iran	PI 429377	0	0	1	R
77	Iran	PI 429373	0	0	3	MS
78	Iran	PI 401399	1	R	1	R
79	Iraq	PI 243956	1	R	1	R
80	Ireland	Clse 106	1	R	1	R
81	Israel	PI 201991	1	R	2	MR
82	Israel	PI 445977	1	R	2	MR
83	Israel	PI 445980	1	R	2	MR
84	Italy	Clse 105	1	R	1	R
85	Italy	PI 534929	2	MR	1	R
86	Japan	Clse 107	1	R	1	R
87	Japan	Clse 108	1	R	2	MR
88	Japan	Clse 109	3	MS	1	R
89	Japan	PI 446020	3	MS	2	MR
90	Kazakhstan	PI 234655	1	R	1	R
91	Kazakhstan	PI 234656	1	R	1	R
92	Kenya	PI 535006	2	MR	1	R
93	Korea, South	Clse 110	3	MS	1	R
94	Latvia	PI 267098	1	R	1	R
95	Latvia	PI 446181	1	R	1	R
96	Lithuania	PI 404227	2	MR	2	MR
97	Lithuania	PI 446123	5	S	3	MS
98	Lithuania	PI 446140	0	0	2	MR
99	Macedonia	PI 344991	4	S	2	MR
100	Macedonia	PI 344998	1	R	2	MR
101	Macedonia	PI 378233	1	R	2	MR
102	Macedonia	PI 378239	1	R	2	MR
103	Macedonia	PI 390369	4	S	1	R
104	Macedonia	PI 420560	0	0	2	MR
105	Mexico	PI 446058	1	R	2	S
106	Mexico	PI 542467	1	R	2	MR
107	Montenegro	PI 344980	3	MS	3.5	MS
108	Montenegro	PI 349912	2	MR	1	R
109	Morocco	PI 525203	2	MR	2	MR
110	Morocco	PI 525205	4	S	4	S
111	Morocco	PI 525207	0	0	2	MR

Table 4.1. continued from previous page

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
112	Netherlands	PI 290425	1	R	4	S
113	Netherlands	PI 315962	2	MR	1	R
114	Netherlands	PI 330445	2	MR	3.5	MS
115	Netherlands	PI 446208	0	0	2	MR
116	Pakistan	PI 218110	1	R	1	R
117	Pakistan	PI 219740	1	R	2	MR
118	Pakistan	PI 219741	1	R	1	R
119	Pakistan	PI 289827	3	MS	1	R
120	Pakistan	PI 410534	1	R	1	R
121	Pakistan	PI 561809	1	R	1	R
122	Pakistan	PI 578092	3	MS	2	MR
123	Pakistan	PI 513214	0	0	3	MS
124	Pakistan	PI 219741	0	0	2	MR
125	Poland	PI 323449	2	MR	1	MS
126	Poland	PI 323454	2	MR	2	MR
127	Poland	PI 338383	1	R	2	MR
128	Poland	PI 446177	2	MR	2	MR
129	Poland	PI 535192	3	MS	1	R
130	Poland	PI 534950	0	0	3.5	MS
131	Poland	PI 446368	0	0	3.5	MS
132	Portugal	PI 446195	3	MS	2	MR
133	Portugal	PI 535083	1	R	1	R
134	Portugal	PI 535094	4	S	3	MS
135	Portugal	PI 535095	0	0	3	MS
136	Portugal	PI 534927	0	0	1	R
137	Romania	PI 306487	1	R	1	R
138	Romania	PI 306495	3	MS	1	R
139	Romania	PI 446245	3	MS	3	MS
140	Romania	PI 534943	3	MS	2	MR
141	Romania	PI 535163	2	MR	2	MR
142	Romania	PI 446244	0	0	3.5	MS
143	Russia	PI 280838	1	R	2	MR
144	Russia	PI 283971	1	R	2	MR
145	Russia	PI 445986	0	0	3	MS
146	Russia	PI 445987	0	0	2	MR
147	Russia	PI 446127	0	0	2	MR
148	Serbia	PI 345000	2	MR	1	R

Table 4.1. continued from previous page

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
149	Serbia	PI 378230	2	MR	1	R
150	Serbia	PI 378231	3	MS	1	R
151	Slovakia	PI 290423	3	MS	2	MR
152	South Africa	PI 330407	4	S	3.7	MS
153	South Africa	PI 330413	2	MR	1	R
154	South Africa	PI 330431	2	MR	1	R
155	Spain	PI 256026	4	S	1	R
156	Spain	PI 323365	2	MR	2	MR
157	Spain	PI 323383	1	R	1	R
158	Sweden	Clse 1	2	MR	2	MR
159	Sweden	Clse 20	4	S	1	R
160	Sweden	PI 330439	3	MS	2	MR
161	Sweden	PI 368157	4	S	3.75	MS
162	Sweden	PI 561674	3	MS	2	MR
163	Switzerland	PI 263561	1	R	2	MR
164	Tajikistan	PI 639328	1	R	2	MR
165	Tajikistan	PI 639336	1	R	1	R
166	Turkey	PI 266975	1	R	1	R
167	Turkey	PI 357067	2	MR	1	R
168	Turkey	PI 357091	1	R	1	R
169	Turkey	PI 543408	2	MR	1	R
170	Turkey	PI 543593	4	S	1	R
171	Turkey	PI 543664	2	MR	1	R
172	Turkey	PI 560572	1	R	1	R
173	Turkey	PI 568106	1	R	1	R
174	Turkey	PI 470297	0	0	3	MS
175	Ukraine	PI 290439	5	S	2	MR
176	Ukraine	PI 372115	3	MS	2	MR
177	Ukraine	PI 534948	2	MR	1	R
178	UK	PI 330526	1	R	1	R
179	UK	PI 345531	2	MR	2	MR
180	UK	PI 414080	1	R	1	R
181	USA	Clse 28	1	R	1	R
182	USA	Clse 38	2	MR	1	R
183	USA	Clse 84	1	R	1	R
184	USA	PI 323377	2	MR	2	MR

Table 4.1. continued from previous page

SN.	Country	Accession No	Race 5		Race 1	
			Lesion Type	Reaction	Lesion Type	Reaction
185	USA	PI 464583	2	MR	4	S
186	USA	PI 491395	3	MS	1	R
187	USA	PI 522185	1	R	3.5	MS
188	USA	PI 534954	3	MS	1	R
189	USA	PI 534961	2	MR	4	S
190	USA	PI 534962	2	MR	2	MR
191	USA	PI 535154	2	MR	2	MR
192	USA	PI 535159	1	R	1	R
193	USA	PI 535199	2	MR	1	R
194	USA	PI 542469	2	MR	1	R
195	USA	PI 543729	2	MR	2	MR
196	USA	PI 552973	2	MR	2	MR
197	USA	PI 559980	4	S	1	R
198	USA	PI 559981	3	MS	1	R
199	USA	PI 628642	2	MR	1	R
200	USA	Clse 174	4	S	2	MR
201	USA	Clse 176	3	MS	1	R
202	USA	Clse 521	3	MS	2	MR
203	USA	PI 619184	1	R	2	MR
204	USA	PI 422425	0	0	1	R
205	USA	PI 445977	0	0	1	R
206	USA	PI 446001	0	0	1	R
207	USA	PI 534936	0	0	1	R
208	USA	PI 534939	0	0	1	R
209	USA	PI 534954	0	0	1	R
210	USA	PI 446266	0	0	3	MS
211	Uruguay	PI 535174	2	MR	1	R

[a] = Due to 211 and 171 rye genotypes insensitivity to Ptr ToxA and Ptr ToxB, respectively, the data was not shown in the table. S = susceptible; MS = moderately susceptible; Plants were rated on lesion type 1-5 scale wherase 1-2 are Moderately resistant to resistant and 3-5 are moderately susceptible to susceptible (Lamari and Bernier (1989). 0 = genotype not tested for race 5.

Table 4.2. List of *P. tritici-repentis* isolates collected and race characterized from rye during 2013-14 in South Dakota

Isolates	Race	*Ptr ToxA*	*Ptr ToxB*	**MT*
13-3-P1.1	Race 4	-	-	+
13-3-P1.2	Race1	+	-	+
13-3-P1.3	Race1	+	-	+
13-3-P1.4	Race1	+	-	+
13-3-P2.1	Race1	+	-	+
13-3-P2.2	Race1	+	-	+
13-3-P2.3	Race1	+	-	+
13-3-P2.4	Race1	+	-	+
13-3-P2.5	Race1	+	-	+
13-3-P3.1	Race1	+	-	+
13-3-P3.2	Race1	+	-	+
13-3-P3.3	Race1	+	-	+
13-3-P4.1	Race1	+	-	+
13-3-P4.2	Race1	+	-	+
13-3-P4.3	Race1	+	-	+
13-3-P4.4	Race1	+	-	+
13-3-P5.1	Race1	+	-	+
13-3-P5.2	Race1	+	-	+
13-3-P5.3	Race1	+	-	+
13-3-P5.4	Race1	+	-	+
14 40-P1.1 NSS	Race1	+	-	+
14- 40-P2	Race1	+	-	+
14- 40-P3	Race1	+	-	+
14-40-P4	Race 4	-	-	+
14-40-P5	Race 4	-	-	+
14 -40-P6	Race 4	-	-	+
14- 40-P7	Race 4	-	-	+
14-41-P1	Race 4	-	-	+
14-41-P2	Race 4	-	-	+
14-41-P3	Race 4	-	-	+
14-41-P4	Race 4	-	-	+
14-41-P5	Race 4	-	-	+
14-41-P6	Race 4	-	-	+
14-41-P7	Race 4	-	-	+
14-41-P8	Race 4	-	-	+
14-41-P9	Race 4	-	-	+
14-41-P10	Race 4	-	-	+
14-41-P10.1	Race 4	-	-	+

Table 4.2. continued from previous page

Isolates	Race	*Ptr ToxA*	*Ptr ToxB*	**MT*
14-41-P14.1	Race 4	-	-	+
14-41-P15.1	Race 4	-	-	+
14- 41-P11	Race 4	-	-	+
14- 41-P11.1	Race 4	-	-	+
14- 41-P12	Race 4	-	-	+
14- 41-P12.1	Race 4	-	-	+
14- 41-P13	Race 4	-	-	+
14- 41-P13.7	Race 4	-	-	+
14-41-P14	Race 4	-	-	+
14-41-P15	Race 4	-	-	+
14 -41-P16	Race 4	-	-	+
15 -41-P16.1	Race 4	-	-	+
14- 41-P17	Race 4	-	-	+
14- 41-P18	Race 4	-	-	+
14- 41-P19	Race 4	-	-	+
14- 41-P20	Race 4	-	-	+
14- 41-P21	Race 4	-	-	+
14- 41-P22	Race 4	-	-	+
14- 41-P23	Race 4	-	-	+
14- 41-P24	Race 4	-	-	+
14- 41-P25	Race 4	-	-	+
14- 41-P26	Race 4	-	-	+
14- 41-P27	Race 4	-	-	+
14 42-P1	Race 4	-	-	+
14 42-P1.3	Race 4	-	-	+
14 42-P2	Race 4	-	-	+
14 42-P3	Race 4	-	-	+
14 42-P4	Race 4	-	-	+
14 42-P4.1	Race 1	+	-	+
14 42-P5	Race 4	-	-	+
14 42-P6	Race 4	-	-	+
14 42-P7	Race 4	-	-	+
14 42-P8	Race 4	-	-	+
14 42-P9	Race 4	-	-	+
14 42-P10	Race 4	-	-	+
14- 42-P11	Race 4	-	-	+
14 42-P12	Race 4	-	-	+
14 42-P13	Race 4	-	-	+

Table 4.2. continued from previous page

Isolates	Race	*Ptr ToxA*	*Ptr ToxB*	**MT*
14 42-P14	Race 4	-	-	+
14 42-P15	Race 4	-	-	+
14 42-P16	Race 4	-	-	+
14 42-P17	Race 4	-	-	+
14 42-P18	Race 4	-	-	+
14 42-P19	Race 4	-	-	+
14 42-P20	Race 4	-	-	+
14 42-P21	Race 4	-	-	+
14 42-P22	Race 4	-	-	+
14-42-P23	Race 4	-	-	+
14-42-P24	Race 4	-	-	+
14-42-P25	Race 4	-	-	+
14-42-P26	Race 4	-	-	+
14-42-P27	Race 4	-	-	+
14-42-P28	Race 4	-	-	+
14-42-P29	Race 4	-	-	+
14-42-P30	Race 4	-	-	+
14-42-P31	Race 4	-	-	+
14-42-P32	Race 4	-	-	+
14-42-P33	Race 4	-	-	+
14-51-P1	Race 4	-	-	+
14-51-P2	Race 4	-	-	+
14-51-P3	Race 4	-	-	+
14-51-P4	Race 4	-	-	+
14-51-P6	Race 4	-	-	+
14-51-P7	Race 4	-	-	+
14-51-P9	Race 4	-	-	+

* Mating type genes specific to *P. tritici-repentis*; + = *Ptr ToxA* gene present; - = *Ptr ToxB* gene absent

Table 5.1(a). Phenotypic and genotypicrace characterization of 138 *P. tritici-repentis* isolates collected from wheat at South Dakota during 2012

No	Isolates (2012)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
1	12-S35-SN48-P1	**1**	**+**	**-**	**+**
2	12-S36-SN49-P1	**1**	**+**	**-**	**+**
3	12-S36-SN49-P21.2	*	**+**	**-**	**+**
4	12-S4-SN4-P1	**1**	**+**	**-**	**+**
5	12-S8-SNF-P1	**1**	**+**	**-**	**+**
6	12-S9-SN9-P1	**1**	**+**	**-**	**+**
7	12-S9-SN9-P2	*	**+**	**-**	**+**
8	12-S10-SN10-P1	**1**	**+**	**-**	**+**
9	12-S43-P1	*	**+**	**-**	**+**
10	12-S43-P2	*	**+**	**-**	**+**
11	12-S43-P3	*	**+**	**-**	**+**
12	12-S43-P4	*	**+**	**-**	**+**
13	12-S43-P5	*	**+**	**-**	**+**
14	12-S43-P6	*	**+**	**-**	**+**
15	12-S43-P7	*	**+**	**-**	**+**
16	12-S43-P8	*	**+**	**-**	**+**
17	12-N11-P1	*	**+**	**-**	**+**
18	12-N11-P2	*	**+**	**-**	**+**
19	12-N11-P4	*	**+**	**-**	**+**
20	12-S35-SN48-P1	*	**+**	**-**	**+**
21	12-N11-P5	*	**+**	**-**	**+**
22	12-N11-P6	*	**+**	**-**	**+**
23	12-N11-P7	*	**+**	**-**	**+**
24	12-N11-P8	*	**+**	**-**	**+**
25	12-S3-P1	*	**+**	**-**	**+**
26	12-S3-P2	**1**	**+**	**-**	**+**
27	12-S3-P3	*	**+**	**-**	**+**
28	12-S3-P3.4	*	**+**	**-**	**+**
29	12-S3-P4	*	**+**	**-**	**+**
30	12-S3-P4.2	*	**+**	**-**	**+**
31	12-S3-P5	*	**+**	**-**	**+**
32	12-S3-P6	*	**+**	**-**	**+**
33	12-S3-P7	*	**+**	**-**	**+**
34	12-S3-P8	*	**+**	**-**	**+**
35	12-S3-P9	*	**+**	**-**	**+**
36	12-S3-P10	*	**+**	**-**	**+**
37	12-S3-P11	*	**+**	**-**	**+**

Table 5.1(a). continued from previous page

No	Isolates (2012)	Race	*Ptr ToxA*	*Ptr ToxB*	[q]*MT*
38	12-S3-P12	*	+	-	+
39	12-S21-P1	*	+	-	+
40	12-S2(L)-P1	*	+	-	+
41	12-S2(s)-P2	*	+	-	+
42	12-S2-P2.2	*	+	-	+
43	12-S2(s)-P3	*	+	-	+
44	12-S2(s)-P4	*	+	-	+
45	12-S2(s)-P5	*	+	-	+
46	12-S2(w)-P6	*	+	-	+
47	12-S2-P7	*	+	-	+
48	12-S2-P8	*	+	-	+
49	12-S2-P9	*	+	-	+
50	12-S2-P10	*	+	-	+
51	12-S2-P11	*	+	-	+
52	12-S2-P12	*	+	-	+
53	12-S2-P13	*	+	-	+
54	12-S2-P14	*	+	-	+
55	12-S2-P15	*	+	-	+
56	12-S1-P1(s)	*	+	-	+
57	12-S1-P2	*	+	-	+
58	12-S1-P3(s)	*	+	-	+
59	12-S1-P4(s)	*	+	-	+
60	12-S1-P5	*	+	-	+
61	12-S1-P6	*	+	-	+
62	12-S9-SN9-P1	*	+	-	+
63	12-S11-SN11-P3.2	*	+	-	+
64	12-S11-SN11-P4.2	*	+	-	+
65	12-S11-SN11-P4.3	*	+	-	+
66	12-S11-SN11-P5.2	*	+	-	+
67	12-S11-SN11-P5.3	*	+	-	+
68	12-S11-SN11-P7.2	*Nec+Chl-*	-	-	+
69	12-S11-SN11-P7.3	**4**	-	-	+
70	12-S11-SN11-P7.4	**4**	-	-	+
71	12-S12-SN12-P1	**4**	-	-	+
72	12-S12-SN12-P2	*Nec+Chl-*	-	-	+
73	12-S12-SN12-P3	*Nec+Chl-*	-	-	+
74	12-S12-SN12-P4	*	-	-	+
75	12-S12-SN12-P5	**4**	-	-	+
76	12-S12-SN12-P1.2	*Nec+Chl-*	-	-	+

Table 5.1(a). continued from previous page

No	Isolates (2012)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
77	12-S12-SN12-P1.3	**4**	-	-	+
78	12-S12-SN12-P2.2	**4**	-	-	+
79	12-S12-SN12-P2.3	**4**	-	-	+
80	12-S12-SN12-P2.4	*Nec+Chl-*	-	-	+
81	12-S12-SN12-P3.3	*Nec+Chl-*	-	-	+
82	12-S12-SN12-P3.4	*	-	-	+
83	12-S12-SN12-P4.2	*	+	-	+
84	12-S12-SN12-P4.3	*	+	-	+
85	12-S12-SN12-P4.4	*	+	-	+
86	12-S12-SN12-P5.2	**4**	-	-	+
87	12-S12-SN12-P5.3	*	+	-	+
88	12-S18-SN18-P1	**1**	+	-	+
89	12-S18-SN18-P15.2	*	+	-	+
90	12-S18-SN18-P15.3	*	+	-	+
91	12-S18-SN18-P15.4	*	+	-	+
92	12-S36-SN49-P21.2	*	-	-	+
93	12-S36-SN49-P21.3	*	-	-	+
94	12-S26-SN27-P1	**1**	+	-	+
95	12-S26-SN27-P1.3	*	+	-	+
96	12-S26-SN27-P1.4	*	+	-	+
97	12-S26-SN27-P2	**2**	+	-	+
98	12-S26-SN27-P2.2	*	+	-	+
99	12-S26-SN27-P2.3	*	+	-	+
100	12-S26-SN27-P2.4	*	+	-	+
101	12-S26-SN27-P2.5	*	+	-	+
102	12-S26-SN27-P3.2	*	+	-	+
103	12-S26-SN27-P3.4	*	+	-	+
104	12-S26-SN27-P4.2	*	+	-	+
105	12-S26-SN27-P4.3	*	+	-	+
106	12-S26-SN27-P4.4	*	+	-	+
107	12-S26-SN27-P5.2	*	+	-	+
108	12-S26-SN27-P5.3	*	+	-	+
109	12-S26-SN27-P5.4	*	+	-	+
110	12-S27-SN28-P1.2	*	+	-	+
111	12-S27-SN28-P1.4	**1**	+	-	+
112	12-S27-SN28-P5.2	*	+	-	+
113	12-S27-SN28-P11.2	*	+	-	+
114	12-S27-SN28-P12.2	*	+	-	+
115	12-S27-SN28-P12.3	*	+	-	+

Table 5.1(a). continued from previous page

No	Isolates (2012)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
116	12-S28-SN29-P1.2	*	+	-	+
117	12-S28-SN29-P2.2	*	-	-	+
118	12-S28-SN29-P2.3	*	+	-	+
119	12-S28-SN29-P2.4	*	+	-	+
120	12-S28-SN29-P3.2	*	+	-	+
121	12-S28-SN29-P3.3	*	+	-	+
122	12-S28-SN29-P3.4	*	+	-	+
123	12-S28-SN29-P4.2	*	+	-	+
124	12-S28-SN29-P4.3	*	+	-	+
125	12-S28-SN29-P5.2	*	+	-	+
126	12-S28-SN29-P5.3	*	+	-	+
127	12-S28-SN29-P5.4	*	+	-	+
128	12-S29-SN29-P2.2	*	+	-	+
129	12-S29-SN29-P2.3	*	+	-	+
130	12-S29-SN29-P3.2	*	+	-	+
131	12-S29-SN29-P3.3	*	+	-	+
132	12-S29-SN29-P3.4	*	+	-	+
133	12-S29-SN29-P4.2	*	+	-	+
134	12-S29-SN29-P1.2	**1**	+	-	+
135	12-S29-SN29-P1.3	*	+	-	+
136	12-S29-SN29-P1.4	*	+	-	+
137	12-S29-SN29-P4	*	+	-	+
138	12-S29-SN29-P6	*	+	-	+

Table 5.1(b). Phenotypic and genotypic race characterization of total 176 *P. tritici-repentis* isolates collected during 2013 from wheat at South Dakota (n=168) and Nebraska (n=8)

No	Isolates (2013)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
1	13-SE-011-P1	**1**	**+**	**-**	**+**
2	13-SE-012-P1.2	**1**	**+**	**-**	**+**
3	13-SE-012-P1.4	*	**+**	**-**	**+**
4	13-SE-012-P2.2	*	**+**	**-**	**+**
5	13-SE-012-P2.3	**1**	**+**	**-**	**+**
6	13-SE-012-P3.1	*	**+**	**-**	**+**
7	13-SE-012-P3.2	*	**+**	**-**	**+**
8	13-SE-012-P3.3	*	**+**	**-**	**+**
9	13-SE-012-P3.4	*	**+**	**-**	**+**
10	13-NE-017-P1.1	**1**	**+**	**-**	**+**
11	13-NE-017-P1.2	**1**	**+**	**-**	**+**
12	13-NE-017-P1.3	*	**+**	**-**	**+**
13	13-NE-017-P1.4	*	**+**	**-**	**+**
14	13-NE-017-P2.1	*	**+**	**-**	**+**
15	13-NE-017-P2.2	*	**+**	**-**	**+**
16	13-NE-017-P2.3	*	**+**	**-**	**+**
17	13-NE-017-P2.4	*	**+**	**-**	**+**
18	13-NE-017-P3.1	**1**	**+**	**-**	**+**
19	13-NE-017-P3.2	*	**+**	**-**	**+**
20	13-NE-017-P3.3	*	**+**	**-**	**+**
21	13-NE-017-P3.4	*	**+**	**-**	**+**
22	13-NE-017-P4.1	*	**+**	**-**	**+**
23	13-NE-017-P4.2	*	**+**	**-**	**+**
24	13-NE-017-P4.3	*	**+**	**-**	**+**
25	13-NE-017-P4.4	*	**+**	**-**	**+**
26	13-NE-017-P5.1	*	**+**	**-**	**+**
27	13-NE-017-P5.2	*	**+**	**-**	**+**
28	13-NE-017-P5.3	*	**+**	**-**	**+**
29	13-NE-017-P5.4	*	**+**	**-**	**+**
30	13-NE-017-P6.2	*	**+**	**-**	**+**
31	13-NE-017-P6.3	*	**+**	**-**	**+**
32	13-101-P1	**1**	**+**	**-**	**+**
33	13-102-P1	**1**	**+**	**-**	**+**
34	13-103-P1	**1**	**+**	**-**	**+**
35	13-103-P8.1	**1**	**+**	**-**	**+**
36	13-103-P8.3	*	**+**	**-**	**+**
37	13-103-P8.5	*	**+**	**-**	**+**
38	13-103-P8.6	*	**+**	**-**	**+**

Table 5.1(b). continued from previous page

No.	Isolates (2013)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
39	13-103-P9.1	*	+	-	+
40	13-103-P9.2	*	+	-	+
41	13-103-P9.3	*	+	-	+
42	13-103-P9.4	**1**	+	-	+
43	13-103-P10.1	*	+	-	+
44	13-103-P10.2	*	+	-	+
45	13-103-P10.3	*	+	-	+
46	13-103-P10.4	*	+	-	+
47	13-103-P10.5	*	+	-	+
48	13-103-P10.6	*	+	-	+
49	13-103-P11.4	*	+	-	+
50	13-103-P11.5	*	+	-	+
51	13-103-P11.6	*	+	-	+
52	13-103-P12.5	*	+	-	+
53	13-103-P13.1	*	+	-	+
54	13-103-P13.2	*	+	-	+
55	13-103-P13.3	*	+	-	+
56	13-103-P13.4	*	+	-	+
57	13-103-P13.5	*	-	-	+
58	13-103-P13.6	*	+	-	+
59	13-104-P1	**1**	+	-	+
60	13-105-P1	**1**	+	-	+
61	13-106-P1	**1**	+	-	+
62	13-NW012-P1.1	**1**	+	-	+
63	13-NW019-P2.1	**1**	+	-	+
64	13-NW22-P2.1	**1**	+	-	+
65	13-NW23-P1.1	**1**	+	-	+
66	13-NW24-P1.1	**1**	+	-	+
67	13-NW24-P6.3	*	+	-	+
68	13-NE018-P1.1	**1**	+	-	+
69	13-103-P4.12	**1**	+	-	+
70	13-103-P4.19	**4**	-	-	+
71	13-103-P4.22	**4**	-	-	+
72	13-103-P4.26	**4**	-	-	+
73	13-103-P4.27	**5**	-	+	+
74	13-103-P4.29	**4**	-	-	+
75	13-103-P4.30	**4**	-	-	+

Table 5.1(b). continued from previous page

No.	Isolates (2013)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
76	13-103-P4.31	**1**	**+**	**-**	**+**
77	13-103-P4.32	**1**	**+**	**-**	**+**
78	13-103-P4.33	**1**	**+**	**-**	**+**
79	13-103-P4.37	**1**	**+**	**-**	**+**
80	13-103-P4.41	**1**	**+**	**-**	**+**
81	13-103-P4.42	**1**	**+**	**-**	**+**
82	13-103-P4.45	**1**	**+**	**-**	**+**
83	13-103-P4.46	**1**	**+**	**-**	**+**
84	13-103-P4.47	**1**	**+**	**-**	**+**
85	13-103-P4.48	**1**	**+**	**-**	**+**
86	13-103-P4.49	**4**	**-**	**-**	**+**
87	13-103-P4.51	**4**	**-**	**-**	**+**
88	13-103-P4.52	**4**	**-**	**-**	**+**
89	13-103-P4.53	**5**	**-**	**+**	**+**
90	13-103-P4.55	**1**	**+**	**-**	**+**
91	13-103-P4.56	**1**	**+**	**-**	**+**
92	13-103-P4.57	**1**	**+**	**-**	**+**
93	13-103-P4.58	*	**+**	**-**	**+**
94	13-101-P2	*	**+**	**-**	**+**
95	13-101-P3	*	**+**	**-**	**+**
96	13-101-P4	*	**+**	**-**	**+**
97	13-101-P5	*	**+**	**-**	**+**
98	13-102-P2	*	**+**	**-**	**+**
99	13-102-P3	*	**+**	**-**	**+**
100	13-102-P4	*	**+**	**-**	**+**
101	13-102-P5	*	**+**	**-**	**+**
102	13-102-P6	*	**+**	**-**	**+**
103	13-102-P7	*	**+**	**-**	**+**
104	13-102-P8	*	**+**	**-**	**+**
105	13-102-P9	*	**+**	**-**	**+**
106	13-102-P10	*	**+**	**-**	**+**
107	13-103-P2	*	**+**	**-**	**+**
108	13-103-P3	*	**+**	**-**	**+**
109	13-103-P4	*	**+**	**-**	**+**
110	13-103-P5	*	**+**	**-**	**+**
111	13-103-P6	*	**+**	**-**	**+**
112	13-103-P7	*	**+**	**-**	**+**
113	13-01-P1	**1**	**+**	**-**	**+**

Table 5.1(b). continued from previous page

No.	Isolates (2013)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
114	13-01-P2	*	+	-	+
115	13-01-P3	*	+	-	+
116	13-01-P4	*	+	-	+
117	13-01-P5	*	+	-	+
118	13-01-P6	*	+	-	+
119	13-01-P7	*	+	-	+
120	13-01-P8	*	+	-	+
121	13-02-P1	**1**	+	-	+
122	13-02-P2	*	+	-	+
123	13-02-P3	*	+	-	+
124	13-02-P4	*	+	-	+
125	13-02-P5	*	+	-	+
126	13-02-P6	*	+	-	+
127	13-02-P7	*	+	-	+
128	13-02-P8	*	+	-	+
129	13-03-P1	**1**	+	-	+
130	13-3-P1.1	**1**	+	-	+
131	13-03-P2	*	+	-	+
132	13-3-P2.1	**1**	+	-	+
133	13-03-P3	*	+	-	+
134	13-3-P3.1	**1**	+	-	+
135	13-03-P4	*	+	-	+
136	13-3-P4.1	**1**	+		
137	13-03-P5	*	+	-	+
138	13-03-P6	*	+	-	+
139	13-03-P7	*	+	-	+
140	13-03-P8	*	+	-	+
141	13-07-P1	**1**	+	-	+
142	13-07-P2	*	+	-	+
143	13-07-P3	*	+	-	+
144	13-07-P4	*	+	-	+
145	13-07-P5	*	+	-	+
146	13-07-P6	*	+	-	+
147	13-07-P7	*	+	-	+
148	13-08-P1	**1**	+	-	+
149	13-08-P2	*	+	-	+
150	13-08-P3	*	+	-	+
151	13-08-P4	*	+	-	+

Table 5.1(b). continued from previous page

No	Isolates (2013)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
152	13-08-P5	*	+	-	+
153	13-08-P6	*	+	-	+
154	13-08-P7	*	+	-	+
155	13-08-P8	*	+	-	+
156	13-09-P1	**1**	+	-	+
157	13-09-P2	*	+	-	+
158	13-09-P3	*	+	-	+
159	13-09-P4	*	+	-	+
160	13-09-P5	*	+	-	+
161	13-09-P6	*	+	-	+
162	13-09-P7	*	+	-	+
163	13-09-P8	*	+	-	+
164	13-09-P9	*	+	-	+
165	13-09-P10	*	+	-	+
166	13-09-P11	*	+	-	+
167	13-09-P13	*	+	-	+
168	13-09-P14	*	+	-	+
169	13-104-P2(Nebraska)	**1**	+	-	+
170	13-104-P3(Nebraska)	**1**	+	-	+
171	13-104-P4(Nebraska)	**1**	+	-	+
172	13-105-P2(Nebraska)	**1**	+	-	+
173	13-105-P3(Nebraska)	**1**	+	-	+
174	13-105-P4(Nebraska)	**1**	+	-	+
175	13-105-P5(Nebraska)	**1**	+	-	+
176	13-105-P6(Nebraska)	**1**	+	-	+

Table 5.1(c). Phenotypic and genotypic race characterization of total 255 *P. tritici-repentis* isolates collected during 2014 from wheat at South Dakota

No	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
1	14-01-P1	*	+	-	+
2	14-01-P2	*	+	-	+
3	14-01-P3	*	+	-	+
4	14-01-P4	*	+	-	+
5	14-01-P8	*	+	-	+
6	14-01-P10	*	+	-	+
7	14-03-P1	*	+	-	+
8	14-03-P4	*	+	-	+
9	14-03-P5	*	+	-	+
10	14-04-P1	*	+	-	+
11	14-04-P2	*	+	-	+
12	14-04-P3	**1**	+	-	+
13	14-04-P4	*	+	-	+
14	14-04-P5	*	+	-	+
15	14-04-P6	**1**	+	-	+
16	14-04-P7	*	+	-	+
17	14-04-P8	*	+	-	+
18	14-04-P9	*	+	-	+
19	14-04-P10	*	+	-	+
20	14-04-P11	*	+	-	+
21	14-04-P12	*	+	-	+
22	14-04-P13	*	+	-	+
23	14-04-P14	*	+	-	+
24	14-04-P15	*	+	-	+
25	14-05-P1	*	+	-	+
26	14-05-P1.5	*	+	-	+
27	14-05-P2	*	+	-	+
28	14-05-P3	*	+	-	+
29	14-05-P4	*	+	-	+
30	14-05-P6	*	+	-	+
31	14-05-P7	*	+	-	+
32	14-05-P8	*	+	-	+
33	14-05-P9	*	+	-	+
34	14-05-P10	*	+	-	+
35	14-05-P11	*	+	-	+
36	14-05-P12	*	+	-	+
37	14-05-P13	*	+	-	+

Table 5.1(c). continued from previous page

No	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[q]*MT*
38	14-05-P14	*	+	-	+
39	14-06-P2	**1**	+	-	+
40	14-06-P3	*	+	-	+
41	14-06-P11	*	+	-	+
42	14-06-P12	*	+	-	+
43	14-06-P13	**1**	+	-	+
44	14-06-P17	*	+	-	+
45	14-09-P2	*	+	-	+
46	14-09-P3	*	+	-	+
47	14-09-P4	*	+	-	+
48	14-09-P5	*	+	-	+
49	14-09-P6	*	+	-	+
50	14-09-P7	*	+	-	+
51	14-09-P8	*	+	-	+
52	14-09-P11	**1**	+	-	+
53	14-09-P12	*	+	-	+
54	14-11-P4	**1**	+	-	+
55	14-11-P5	**1**	+	-	+
56	14-11-P6	*	+	-	+
57	14-11-P7	*	+	-	+
58	14-11-P8	*	+	-	+
59	14-11-P9	*	+	-	+
60	14-11-P10	*	+	-	+
61	14-11-P11	*	+	-	+
62	14-11-P12	*	+	-	+
63	14-11-P13	*	+	-	+
64	14-11-P14	**1**	+	-	+
65	14-11-P16	*	+	-	+
66	14-11-P17	*	+	-	+
67	14-11-P18	*	+	-	+
68	14-13-P3	*	+	-	+
69	14-13-P4	*	+	-	+
70	14-13-P5	*	+	-	+
71	14-13-P6	*	+	-	+
72	14-13-P9	*	+	-	+
73	14-13-P14	*	+	-	+
74	14-13-P18	*	+	-	+

Table 5.1(c). continued from previous page

No	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	*[a]MT*
75	14-14-P1	*	+	-	+
76	14-14-P2	1	+	-	+
77	14-14-P3	1	+	-	+
78	14-14-P4	*	+	-	+
79	14-14-P5	*	+	-	+
80	14-14-P6	*	+	-	+
81	14-14-P7	*	+	-	+
82	14-14-P8	*	+	-	+
83	14-14-P10	*	+	-	+
84	14-14-P11	*	+	-	+
85	14-14-P12	*	+	-	+
86	14-14-P13	*	+	-	+
87	14-14-P14	*	+	-	+
88	14-14-P15	*	+	-	+
89	14-14-P16	*	+	-	+
90	14-14-P17	*	+	-	+
91	14-15-P1	*	+	-	+
92	14-15-P7	1	+	-	+
93	14-15-P8	*	+	-	+
94	14-15-P9	*	+	-	+
95	14-15-P10	*	+	-	+
96	14-15-P13	1	+	-	+
97	14-15-P15	1	+	-	+
98	14-16-P1	*	+	-	+
99	14-16-P2	*	+	-	+
100	14-16-P3	*	+	-	+
101	14-16-P4	*	+	-	+
102	14-16-P5	*	+	-	+
103	14-16-P6	*	+	-	+
104	14-16-P7	*	+	-	+
105	14-16-P8	*	+	-	+
106	14-16-P9	1	+	-	+
107	14-16-P10	*	+	-	+
108	14-16-P11	*	+	-	+
109	14-22-P1	*	+	-	+
110	14-22-P2	1	+	-	+
111	14-22-P3	*	+	-	+
112	14-22-P4	1	+	-	+

Table 5.1(c). continued from previous page

No.	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[q]*MT*
113	14-22-P5	**1**	+	-	+
114	14-22-P6	**1**	+	-	+
115	14-22-P7	*	+	-	+
116	14-22-P8	*	+	-	+
117	14-22-P9	**1**	+	-	+
118	14-22-P10	*	+	-	+
119	14-22-P11	*	+	-	+
120	14-22-P12	*	+	-	+
121	14-22-P14	**1**	+	-	+
122	14-22-P15	*	-	-	+
123	14-22-P17	**1**	+	-	+
124	14-43-P4	**4**	-	-	+
125	14-16-P10	*	+	-	+
126	14-28-P1	**1**	+	-	+
127	14-28-P2	*	+	-	+
128	14-28-P3	*	+	-	+
129	14-28-P4	**1**	+	-	+
130	14-28-P5	*	+	-	+
131	14-28-P6	*	+	-	+
132	14-28-P7	*	+	-	+
133	14-28-P8	*	+	-	+
134	14-28-P9	*	+	-	+
135	14-29-P1	*	+	-	+
136	14-29-P2	*	+	-	+
137	14-29-P3	**1**	+	-	+
138	14-29-P4	**1**	+	-	+
139	14-29-P5	**1**	+	-	+
140	14-29-P6	**1**	+	-	+
141	14-29-P7	**1**	+	-	+
142	14-29-P8	**1**	+	-	+
143	14-29-P9	**1**	+	-	+
144	14-29-P10	*	+	-	+
145	14-29-P11	*	+	-	+
146	14-29-P12	*	+	-	+
147	14-29-P13	*	+	-	+
148	14-29-P14	*	+	-	+
149	14-29-P15	*	+	-	+
150	14-29-P16	*	+	-	+
151	14-29-P17	*	+	-	+

Table 5.1(c). continued from previous page

No.	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[q]*MT*
152	14-29-P18	*	+	-	+
153	14-29-P19	*	+	-	+
154	14-29-P20	*	+	-	+
155	14-29-P21	*	+	-	+
156	14-NE	*	+	-	+
157	14-43-P1	*	-	-	+
158	14-43-P3	**4**	-	-	+
159	14-44-P1	*	+	-	+
160	14-44-P2	*	+	-	+
161	14-45-P2	*	+	-	+
162	14-45-P3	*	+	-	+
163	14-45-P4	*	+	-	+
164	14-45-P6	*	+	-	+
165	14-45-P7	*	+	-	+
166	14-45-P8	*	+	-	+
167	14-49-P1.2	*	+	-	+
168	14-49-P1.3	*	+	-	+
169	14-49-P1.4	*	+	-	+
170	14-49-P1.5	*	+	-	+
171	14-49-P1.6	*	+	-	+
172	14-49-P1.7	*	+	-	+
173	14-49-P1.8	*	+	-	+
174	14-49-P2.1	*	+	-	+
175	14-49-P2.2	*	+	-	+
176	14-49-P2.3	*	+	-	+
177	14-49-P2.4	*	+	-	+
178	14-49-P2.5	*	+	-	+
179	14-49-P2.6	*	+	-	+
180	14-49-P2.7	*	+	-	+
181	14-49-P2.8	*	+	-	+
182	14-49-P3.1	*	+	-	+
183	14-49-P3.2	*	+	-	+
184	14-49-P3.3	*	+	-	+
185	14-49-P3.4	*	+	-	+
186	14-49-P3.5	*	+	-	+
187	14-49-P3.6	*	+	-	+
188	14-49-P3.7	*	+	-	+
189	14-49-P4.1	*	+	-	+
190	14-49-P4.2	*	+	-	+

Table 5.1(c). continued from previous page

No.	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
191	14-49-P4.3	*	+	-	+
192	14-49-P4.4	*	+	-	+
193	14-49-P4.5	*	+	-	+
194	14-49-P4.6	*	+	-	+
195	14-49-P4.7	*	+	-	+
196	14-49-P4.8	*	+	-	+
197	14-49-P5.1	*	+	-	+
198	14-49-P5.2	*	+	-	+
199	14-49-P5.3	*	+	-	+
200	14-49-P5.4	*	+	-	+
201	14-49-P5.5	*	+	-	+
202	14-49-P5.6	*	+	-	+
203	14-49-P6.1	*	+	-	+
204	14-49-P6.2	*	+	-	+
205	14-49-P6.3	*	+	-	+
206	14-49-P6.4	*	+	-	+
207	14-49-P6.5	*	+	-	+
208	14-49-P6.6	*	+	-	+
209	14-49-P6.7	*	+	-	+
210	14-49-P11.9	*	+	-	+
211	14-49-P42	*	+	-	+
212	14-50-P1.1	*	+	-	+
213	14-50-P1.2	*	+	-	+
214	14-50-P1.3	*	+	-	+
215	14-50-P1.4	*	+	-	+
216	14-50-P1.6	*	+	-	+
217	14-50-P1.7	*	+	-	+
218	14-50-P2.1	**4**	-	-	+
219	14-50-P2.2	*	-	-	+
220	14-50-P2.3	*	-	-	+
221	14-50-P2.4	*	-	-	+
222	14-50-P2.5	*	-	-	+
223	14-50-P2.6	*	-	-	+
224	14-50-P2.7	**4**	-	-	+
225	14-50-P3.1	**4**	-	-	+
226	14-50-P3.2	**4**	-	-	+
227	14-50-P3.5	*	-	-	+
228	14-50-P3.6	**4**	-	-	+
229	14-50-P3.7	*	-	-	+

Table 5.1(c). continued from previous page

No.	Isolates (2014)	Race	*Ptr ToxA*	*Ptr ToxB*	[a]*MT*
230	14-50-P4.1	*	-	-	+
231	14-50-P4.3	**4**	-	-	+
232	14-50-P4.4	*	-	-	+
233	14-50-P4.5	**4**	-	-	+
234	14-50-P4.6	*	-	-	+
235	14-50-P4.8	*	-	-	+
236	14-50-P5.1	*	-	-	+
237	14-50-P5.2	*	-	-	+
238	14-50-P5.3	*	-	-	+
239	14-50-P5.4	*	-	-	+
240	14-50-P5.5	*	-	-	+
241	14-50-P5.6	*	-	-	+
242	14-50-P5.7	*	-	-	+
243	14-50-P9.1	*	+	-	+
244	14-50-P9.2	*	+	-	+
245	14-50-P10.1	*	+	-	+
246	14-50-P10.2	*	+	-	+
247	14-50-P10.7	*	+	-	+
248	14-100-P1.3	**1**	+	-	+
249	14-100-P1.2	**1**	+	-	+
250	14-100-P1.1	**1**	+	-	+
251	14-100-P2.1	**1**	+	-	+
252	14-100-P2.2	**1**	+	-	+
253	14-100-P1.4	**1**	+	-	+
254	14-100-P2.3	**1**	+	-	+
255	14-100-P2.4	**1**	+	-	+

Nec+Chl- = Isolates lack in *Ptr ToxA* gene but produce necrosis on Ptr ToxA sensitive wheat differential Glenlea; [a] = *P. tritici-repentis* specific mating gene was amplified; * = Isolates were not characterized on tan spot wheat differentials set; + = *Ptr ToxA*/ *ToxB*/*mating type* gene present and - = *Ptr ToxA*/ *ToxB*/*mating type* gene not present.